Treatment of Pentachlorophenol (PCP) by Integrating Biosorption and Photocatalytic Oxidation

by

CHAN Shuk Mei

A Thesis Submitted in Partial Fulfillment
of the Requirements for the
Degree of Master of Philosophy

in

Biology

July, 2002


香港中文大學
大學圖書館系統
07 MAR 2003
THE CHINESE UNIVERSITY OF HONG KONG

THESIS COMMITTEE:

Prof. P.K. Wong (Supervisor)

Prof. D. Ow (External Examiner)

Prof. L.M. Chu (Internal Examiner)

Prof. S.W. Chiu (Internal Examiner)

Acknowledgements

At the beginning of this thesis, I would like to take an opportunity to acknowledge those people who had given me support during my graduate study.

First of all, I would like to express my gratitude to Prof. P.K. Wong, my supervisor, for his invaluable suggestions, encouragement and support in all the ways of my research as well as critical review of the manuscript.

And I am greatly indebted to Prof. S.W. Chiu and Prof. L.M. Chu, the internal examiners, for giving valuable comments on my research and their attentive evaluation on this thesis. In addition, I would also like to appreciate Prof. D. Ow serving as my external examiner and giving valuable comments on my thesis.

It is also my pleasure to deeply thank Dr. Steven Ng for his valuable comment and support during the study period.

My appreciation is extended to Mr. Patrick C.M. Tang, Thomas Tang, William H.C. Ho, and Ms. Jessie P.K. Lee for their technical assistance. Moreover, I am also grateful to friends in the laboratory, who include Ms. Connie Chan, Ho-yan Chung, Hui-wai Chan, Lopaka Ma, Vivian Pang, Mr. Alex Wong, Colin Lau, Joey Chan, Pui Wang and many others, for their spiritual support and enthusiasm.

Special thanks are also given to Ms. Coty Tsui, Shirley Fong, Mr. Colin Lau and Ho-yin Yip. Their advice is important in smoothing my laboratory work and thesis writing.

Last but not least I would like to express my greatest gratitude to my family for their continuous support.

Abstract

Chitin and chitosan were well-recognized in many previous studies as effective biosorbents to remove a wide range of pollutants such as metal ions, dyes and organic compounds. These studies always focused on the utilization of extracted pure chitin or chitosan. In the present study, the pentachlorophenol (PCP) removal efficiencies by them as well as the crude chitin, a ground product of shrimp shell (*Penaeus japonicus*) consisted of 71% of chitin (chitin A), were compared.

In accordance with the results in the present study, the biosorption was influenced by altering the parameters of biosorbent concentration, retention time, pH, temperature and initial pentachlorophenol (PCP) concentration. In general, for all three biosorbents, higher amount of biosorbent gave higher removal efficiency (RE) but lower removal capacity (RC) as more binding sites are available for PCP. The biosorption was described as biphasic mechanism with the first rapid step followed by slow sorption. The apparent-equilibrium was found in the first 60 min. And the PCP removal was enhanced by lowering pH since neutral PCP was favorable for absorption. In addition, the higher temperature regressed the biosorption efficiency. It indicated that the adsorption might be due to the exothermic force such as hydrogen bonding. Meanwhile, the binding sites of chitin B (91% pure chitin) and chitosan reached saturation in the range of PCP concentrations (5-300 mg/L). But this phenomenon was not apparent in chitin A.

On the contrary, by considering the two monolayer adsorption models, Langmuir and Freundlich isotherms, the adsorption might be homogeneous, as the correlation coefficient from Langmuir model was higher. And chitin A showed the highest capacity and affinity for PCP compounds, followed by chitosan and chitin B. Therefore, chitin A was introduced as a better PCP biosorbent compared with pure chitin and chitosan. It was not only due to the higher efficiency, but also the more economic with less treatment process.

To thoroughly remediate PCP, photocatalytic oxidation (PCO) was also employed after the biosorption. The semi-liquid phase of PCP, that was PCP

adsorbed on chitin A and suspended in aqueous media, was feasible for PCO degradation. One hundred % of PCP removal was achieved after 4 h irradiation time, in 100 mL solution with 6.7 mM of H_2O_2 and 200 mg/L of TiO_2. On the other hand, the biosorbent was resistant to PCO and had no change in chitin content, functional groups and biosorption efficiency except protein content after PCO. But the removal of protein did not influence the adsorption efficiency of biosorbent. Therefore it then could be used for multiple PCP biosorption and PCO cycles and lower the treatment cost.

The intermediates of PCP was identified as 2,3,5,6-tetrachlorohydroquinone (TeHQ) and 2,3,5,6-tetrachlorophenol ($C_6H_2Cl_4O$) by GC/MS analysis. In addition, the toxicity of sample was monitored by the solid-phase Microtox® test, which showed decreasing along the irradiation time. Therefore, the combination of biosorption and PCO treatment was feasible for PCP remediation.

摘要

甲殼素 (又名幾丁質，Chitin) 及殼聚糖 (又名幾丁聚醣，Chitosan) 可以有效地吸附廢水中多種污染物，包括金屬離子、染料及一些有機化合物等。多項研究主要是利用經提煉出來的高純度甲殼素或殼聚糖來作吸附劑。有見及此，本研究利用這兩種生物吸附劑 (biosorbent)，及一種直接由蝦殼磨碎而成未經提煉且含 71%甲殼素的吸附劑—甲殼素甲 (Chitin A) 作研究，比較它們對五氯酚(pentachlorophenol)的吸附功能。

研究結果表明，五氯酚的吸附作用會受多種外在環境因素影響，包括吸附劑份量、吸附時間、溶液酸鹼度、溶液溫度及濃度。概括來說，這三種生物吸附劑對外在環境轉變，都有一致的表現。首先，吸附劑份量增加，能爲五氯酚帶來更多吸附位置，而增加五氯酚的整體吸附效率(removal efficiency)；但降低它的吸附容量(removal capacity)。這個吸附作用包含了兩個階段：快速吸附階段及慢速吸附階段；而表面平衡狀態出現於首六十分鐘。另外，溶液的酸鹼度減少，會使五氯酚形成中性份子的份量增加，從而增加五氯酚的吸附。但溫度提高，會限制吸附量，這顯示出五氯酚及甲殼素吸附劑的吸附作用可能是一種吸熱反應，例如牽涉氫鍵結合。另一方面，在實驗中五氯酚濃度 (5-300 mg/L)，甲殼素乙 (Chitin B，91%甲殼素純度)及殼聚糖都表現出飽和狀態；但甲殼素甲則尚未出現此情況。

要概括出這種吸附作用的特性，可利用兩個單分子層吸附模型：蘭格繆爾模型 (Langmuir model)及費羅因德利希模型 (Freundlich model)。首先，這三種吸附劑之蘭格繆爾等溫線的互相關係系數比費羅因德利希的高，這顯示出這種吸附作用較接近蘭格繆爾模型所描述的均質性吸附。而且，從模型的不變數可見，甲殼素甲的吸附容量及取向都是最高的，接著是殼聚糖，最低是甲殼

素乙。因此，從經濟及實用的角度來看，這種未經純化的甲殼素是一種比已純化的甲殼素或殼聚糖更優越的吸附劑。

要達到徹底處理五氯酚的效果，還要利用光催化作用來將被吸附的五氯酚降解。懸浮在水中的半液態五氯酚(即被吸附在甲殼素甲的五氯酚)，能有效地被催化降解。實驗証明，在 100 mL 含有 6.7 mM 過氧化氫 (H_2O_2)及 200 mg/L 二氧化鈦(TiO_2)的五氯酚溶液中，五氯酚能在四小時紫外光照射後被完全降解。另一方面，除了其蛋白質外，這種吸附劑的甲殼素成份及功能團都被証實可以抵禦光催化作用，使其對五氯酚的吸附效率不變，而吸附劑中蛋白質的降解，亦顯不出其對吸附效能有任何影響。因此，這種生物吸附劑可以作多循環使用，以減低處理成本。

利用 GC/MS 作分析，發現五氯酚的中間產品包括 2,3,5,6-四氯輕基醌(2,3,5,6- tetrachlorohydroquinone)及 2,3,5,6-四氯酚(2,3,5,6-tetrachlorophenol)。而且，從固態 Microtox® test 結果可見，樣本的毒性隨著紫外光的照射時間延長而減低。由此可見，綜合吸附作用及光催化作用來處理五氯酚，是一個可行的方法。

Contents

List of Figures

List of Plates

List of Tables

Abbreviations

A^-	The ionic species
A_b	The amount of PCP extracted from biosorbent (mg)
AIM	Alkaline insoluble materials
ANOVA	Analysis of variance
A_o	The amount of PCP adsorbed on biosorbent (mg)
AOPs	Advanced oxidation processes
A_s	The amount of PCP in solution (mg)
b	The adsorption affinity constant related to energy of adsorption (L/mg)
C_e	The equilibrium concentration of solute in bulk aqueous phase after adsorption (mg/L)
C_o	The initial concentration of PCP in solution (mg/L)
C_t	The concentration of PCP in solution at time t (mg/L)
DA	Degree of *N*-acetylation
DC	Degradation capacity (mg of PCP/g of biosorbent)
DDA	Degree of *N*-deacetylation
DE	Degradation efficiency (%)
DRFT-IR	Diffuse reflectance Fourier transform infra-red
EC50	Median effective concentration
EE	Extraction efficiency (%)
EU	European Union
GC/MS	Gas chromatography/mass spectrometry
GF/C	Glass microfibre filter
HA	The uncharged species
HIOCs	hydrophobic ionizable organic compounds
HKSAR	Hong Kong Special Administrative Region
HNOCs	hydrophobic non-ionizable organic compounds
HPLC	High performance liquid chromatography
ICPI	International Chitin Production Inc.
k	Empirical constant of Freundlich equation, indicative of adsorption capacity
$\log P_{ow}$	Logarithm of octanol-water partition coefficient

n	Empirical constant of Freundlich equation, indicative of Adsorption intensity
NaPCP	Sodium pentachlorophenolate (NaC_6Cl_5O)
o	Octanol phases
OPP	Office of Pesticide Programmes
PAN	Pesticide Action Network
PCDDs	Polychlorinated dibenzo- ρ -dioxins
PCDFs	Polychlorinated-dibenzofurans
PCO	Photocatalytic oxidation
PCP	Pentachlorophenol
pzc	Point of zero charge
pK_a	Dissociation constant
q_e	The amount of solute adsorbed per unit dry weight of biosorbent at concentration C_e (mg of PCP/g of biosorbent)
q_{max}	The theoretical maximum uptake of solute per unit dry weight of biosorbent (mg of PCP/g of biosorbent)
r^2	Linear regression
RC	Removal capacity (mg of PCP/g of biosorbent)
RE	Removal efficiency (%)
SEM	Scanning electron microscope
TeBQ	Ttetrachlorobenzoquinone
TeCP	Tetrachlorophenol
TeHQ	Tetrachlorohydroquinone
THBQ	Trichlorohyrdrobenzoquinone
TOC	Total organic carbon
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
V	The volume of PCP solution (L)
W	The dry weight of biosorbent (g)
w	Water phases
W_b	The dry weight of biosorbent (g)
W_{c+f}	The dry weight of filter paper loaded with chitin residue (g)
W_f	The dry weight of filter paper (g)

1. Introduction

1.1 Pentachlorophenol

There is recent interest in studying the remediation of pentachlorophenol (PCP) after well understanding on its nature, wide application, toxicity and effect to the environment. In the present study, PCP is chosen as a model compound for remediation. The characteristics and other information of PCP are given in following sections.

1.1.1 Characteristics of pentachlorophenol

Pentachlorophenol (Figure 1.1) is a synthetic chemical, which is usually produced by the stepwise-chlorination of phenols in the presence of a catalyst (e.g. anhydrous aluminum chloride or ferric chloride) or the alkaline hydrolysis of hexachlorobenzene (Environmental Health Criteria, 1987; Agency for Toxic Substances and Disease Registry, 1994). Pure PCP is a colorless crystal. However, the commercial grade typically contains sole 86% purity. The impure form, usually contaminated by other polychlorinated phenols such as polychlorinated dibenzo--dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), occurs as dark gray to brown dust, beads or flakes (Environmental Health Criteria, 1987; Agency for Toxic Substances and Disease Registry, 1994; Litchfield & Rao, 1998). PCP has a sharp odor when heated, but very little smell at room temperature (Agency for Toxic Substances and Disease Registry, 1994).

The physico-chemical properties of PCP are listed in Table 1.1 (Agency for Toxic Substances and Disease Registry, 1994). Its high boiling point and low vapor pressure suggest PCP is not readily volatile and not likely to escape into atmosphere. In addition, it is a weak acid with low pK_a, so that PCP exists mainly in non-polar form (non-ionic form) at pH < 4.74 (Equation 1.1) (Mills & Hoffmann, 1993; Ning *et al.*, 1999). But when pH is greater than 4.74, most PCP appears as polar form (anionic form) (Muir & Eduljee, 1999). Furthermore, the water solubility is low (Gremaud & Turesky, 1997), but it is very soluble in organic solvents such as alcohol and ether (Litchfield & Rao, 1998). Also it has a higher solubility at alkaline pH as it can dissociate into ionic form, phenolate ion (Equation 1.1) (Environmental Health Criteria, 1987; Christodoulatos & Mohiuddin, 1996; Skurlatov *et al.*, 1997). Therefore, NaOH is usually applied to dissolve PCP (Jacobsen *et al.*, 1996; Slaney &

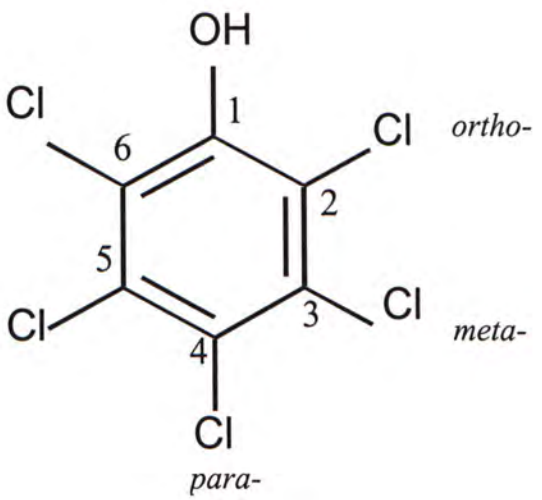


Figure 1.1 Chemical structure of pentachlorophenol (Environmental Health Criteria, 1987; Agency for Toxic Substances and Disease Registry, 1994).

Table 1.1 The physico-chemical properties of pentachlorophenol (modified from Agency for Toxic Substances and Disease Registry, 1994; Muri & Eduljee, 1999).

Property	
Formula	C_6HCl_5O
Molecular weight	266.35
Melting point	190°C
Boiling point	309-310°C
Density	1.978 g/mL at 22°C
Odor	Very pungent
Water solubility	14 mg/L at 20°C in water
Vapour pressure	0.00011 mmHg at 25°C
pK_a	4.74 at 20°C

Bhamidimarri, 1998). The dissociation of PCP is facilitated by withdrawing electrons from the aromatic ring by ringed-chlorine.

$$C_6HCl_5O \rightleftharpoons C_6Cl_5O^- + H^+ \quad (1.1)$$

$$C_6Cl_5O^- + Na^+ \rightleftharpoons Na\,C_6Cl_5O \quad (1.2)$$

With the presence of Na^+, NaPCP (NaC_6Cl_5O) is formed (Equation 1.2), which is between 5,000 and 25,000 times more water-soluble than PCP (Muir and Eduljee, 1999).

1.1.2 Application of pentachlorophenol

PCP is toxic compound which can be used as herbicide, algicide, defoliant, wood preservative, germicide, fungicide, molluscicide and as an ingredient in antifouling paint (Cirelli, 1978; Pignatello *et al*, 1983; Smejtek & Wang, 1993; Danis *et al.*, 1998; Litchfield & Rao, 1998; Slaney & Bhamidimarri, 1998; Stringer & Johnston, 2001). Due to the high toxicity (see Section 1.1.4) and the potential for adverse effects on man and the environment, the use of PCP as pesticide ingredient was restricted in the European Union (EU) (i.e., Austria, Belgium, Finland, Germany, the Netherlands and Sweden) in 1991 (Litchfield & Rao, 1998). But before the restriction, it has been widely used worldwide due to its low cost and non-specific toxicity towards different organisms (Ernestova *et al.*, 1997; Cauntú *et al.*, 2000; Stringer & Johnston, 2001). United States consumption of PCP was reported to be 12.7 kiloton in 1986 and global production was estimated at 25 kiloton in 1989 (Stringer & Johnston, 2001). And it was reported that the EU-consumption was 2,500 tons in 1980s to 426 tons in 1996 (Muir & Eduljee, 1999). On the contrary, it is still allowed to be used in some industrial applications including wood preservation for power line poles, railroad ties, cross arms, and fence posts; impregnating fibers and heavy-duty textiles; synthesizing and processing agents in industrial processes; and treatment of buildings of cultural and historic interest. In 1999, there were still $2x10^7$ pounds of PCP manufactured or processed in United States (derived from Toxics Release Inventory 96, 1999). In addition, the average PCP concentration in ditches near wood treatment site and PCP degrading activated sludge reactors were found to be 500 and 12 mg/L respectively (Jacobsen *et al.*,

1996; Muir & Eduljee, 1999).

1.1.3 The fate of pentachlorophenol in environment

The fate of PCP in the environment is of great importance as it can influence the environment by its toxic, recalcitrant and bioaccumulating effects (Chapman *et al.*, 1982; Jianlong *et al.*, 2000). PCP enters the environment in different ways, e.g. illegal discharge or accidental spilling from factories, flooding or evaporation from treated wood surfaces and pesticides, etc. (Pignatello *et al*, 1983; Danis *et al.*, 1998). As mentioned before, the physical and chemical properties of PCP suggest that not much will evaporate into atmosphere. Most of it will move with water and generally stick to soil particles or sediment (You & Liu, 1996). Therefore, the presence of PCP can be detected in freshwater environment, freshwater sediment, marine and estuarine environment, marine sediment as well as some industrial discharge (Chapman *et al.*, 1982; Agency for Toxic Substances and Disease Registry, 1994; Danis *et al.*, 1998; Muir & Eduljee, 1999; Ning *et al.*, 1999; Cauntú *et al.*, 2000). In addition, the compound can be present and bioaccumulated in fish or other species used for food (Agency for Toxic Substances and Disease Registry, 1994; Mollah & Robinson, 1996a; Stinger & Johnston, 2001).

A statistical expression, predicted no-effect concentration (PNEC), is used to establish whether the substance under consideration (PCP in this case) poses a risk to harm the environment (Muir and Eduljee, 1999). The data are listed in Table 1.2. It is defined that if the measured PCP concentration of the samples taken from different environments is greater than PNEC, it is considered to be concerned that it could pose a risk to the environment. Muir and Eduljee (1999) monitored the PCP concentration in marine waters, marine sediments, freshwater and freshwater sediment as well as suspended matter in countries of European Union. It was found that the freshwater environment contamination in France was the most serious and it was higher than the PNEC. There were 3.3 μg/L, 63 and 179 μg/Kg of PCP presented in freshwater, freshwater sediment and suspended matter respectively in 1996 (Muir & Eduljee, 1999).

The movement of PCP in soils or sediment depends on the acidity (Christodoulatos & Mohiuddin, 1996). It can release into the water body in neutral or alkaline condition, which can be explained by the low value of pK_a (4.74). It is

Table 1.2 Predicted no effect concentrations (PNEC) of different environmental conditions (Mui & Eduljee, 1999).

	Marine waters (μg/L)	Marine sediments (μg/Kg)	Freshwaters (μg/L)	Freshwater sediments/ suspended matter (μg/Kg)
PCP	1.0	25	1.0	25/15
NaPCP	1.0	25	1.0	25/15

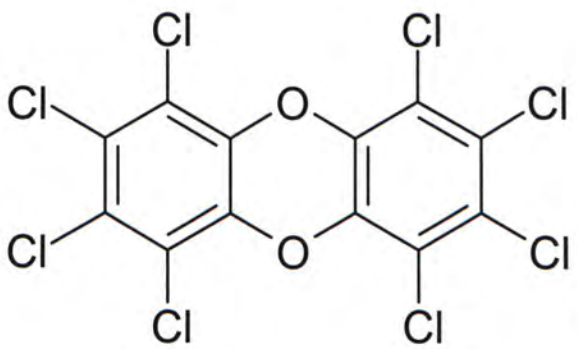
reported that 99% of PCP appears as ionic form at pH 6.7 (Agency for Toxic Substances and Disease Registry, 1994; Jacobsen *et al.*, 1996; Hu *et al.*, 1998; Viraraghavan & Slough, 1999; Jianlong *et al.*, 2000; Stringer & Johnston, 2001); whereas the ionic form is much more easily to desorb from the soils and move into the water body. It is noteworthy that pH of the marine water and freshwater are around 7.2-8.0 (Muir & Eduljee, 1999). That is, most PCP appears as ionic form in marine water or freshwater and readily desorbs from the soils.

PCP, mainly in soils, can be degraded by some kind of microorganisms such as *Arthrobacter* and *Pseudomonas* bacteria, under anaerobic or aerobic conditions (Agency for Toxic Substances and Disease Registry, 1994; Litchfield & Rao, 1998). The intermediate metabolites are varied depending on the species of bacteria and physical conditions such as pH, dissolved oxygen and light; whereas trichlorophenol, tetrachlorophenol, tetrachlorohydroquinone and dichloro-hydroquinone can be included (Reiner *et al.*, 1978; Litchfield & Rao, 1998). Unfortunately, it is postulated that the chlorines at the 3 or 5 meta position were sterically hindered from enzymatic dehalogenation or oxidation (Litchfield & Rao, 1998). Thus, the breakdown products are resistant to be biodegraded and they stay in environment for a long time before complete mineralization into carbon dioxide and water.

Moreover, some PCP may undergo photo-induced condensation or photolysis under sunlight or ultraviolet (UV) (Mollah & Robinson, 1996). Then some highly toxic compounds such as octachlorodibenzo-ρ-dioxin (OCDD) and octachlorodibenzofuran (OCDF) (structures shown in Figure 1.2) are formed as a result of condensation (Agency for Toxic Substances and Disease Registry, 1994; Litchfield & Rao, 1998; Fong, 2001). For photolysis, the degraded products include tetrachlorophenols, three tetrachlorodiols and their respective quinines, chloranilic acid, and eventually 2,3-dichlormaleic acid (Wong and Crosby, 1978).

As a conclusion, PCP, either in freshwater or in marine water environments, could undergo different processes. Less than 15% of PCP is adsorbed on sediment and uptaken by living organisms; 5-28% would undergo photolysis; and 26-46% is biologically degraded after 3 weeks from the introduction of PCP (Pignatello *et al*, 1983; Stringer & Johnston, 2001).

(a)



(b)

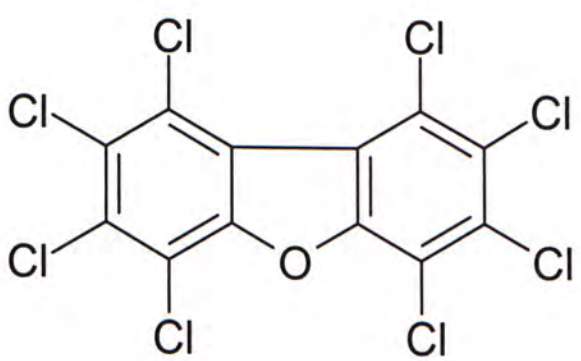


Figure 1.2 Chemical structures of (a) octachlorodibenzo-ρ-dioxin and (b) octachlorodibenzofuran (Agency for Toxic Substances and Disease Registry, 1994; Litchfield & Rao, 1998).

1.1.4 The toxicity of pentachlorophenol

Due to the wide use of PCP, many studies have been performed to investigate its toxic effects towards different species. Table 1.3 summarizes the toxicity of PCP on different species (TerraBase Inc., 2000). Based on the results of oral LD50, PCP is classified as category I, highly acute toxic, inert ingredient in pesticide by USEPA with the acute toxicity rankings system (Ma *et al.*, 2002b). Table 1.4 shows the criteria of pesticide ranking organized by USEPA (Ma *et al.*, 2002b).

PCP is highly toxic because of its interrupt nature to the organisms' metabolism. Litchfield & Rao (1998) investigated the toxic effects of PCP on different kinds of microorganisms. They discovered that PCP inhibited the transport of manganese, amino acid, proline and glycine in *Bacillus subtilis*. And it was found to be an inhibitor of both oxidative phosphorylation and substrate-level phosphorylation in *Streptococcus agalactiae*, and inhibitor of electron transport phosphorylation coupled to fumarate reduction in *Vibrio succinogenes*. Eventually, PCP predominated resulting in poor growth of microorganisms (Litchfield & Rao, 1998).

Meanwhile, similar physiological effects can be observed in animals. PCP can enter the animal body by the route of administration, skin contact, breathing and oral intake. PCP interrupts the respiration process of animals as well as the energy metabolism by altering the enzyme, ATPase and galactosidase activities, and uncoupling oxidative phosphorylation by making cell membranes permeable to protons. As a result, it enhances the consumption rate of oxygen and lipid to generate less energy for maintenance and growth (Muir & Eduljee, 1999). In addition, PCP is proved to be foetotoxic, which influences the reproduction and fetal development of the animals (Environmental Health Criteria, 1987). On the other hand, USEPA evaluates the carcinogenic effect of PCP to humans and laboratory animals, and ranks it as a group B2 teratogen, probable human carcinogen, according to a list of chemical evaluated for carcinogenic potential introduced in 1996 (Cauntú *et al.*, 2000; Ma *et al.*, 2002a). The category is listed in Table 1.5.

It is worth to note that the number and position of chlorines on the aromatic ring affect the toxicity effect. The higher degree of chlorination, the more toxic the compound is (Litchfield & Rao, 1998; Fong, 2001). And, as a rule of thumb,

Table 1.3 The toxicity of PCP on different species (TerraBase Inc., 2000)

Test organisms	Toxicity (mg/L)
Bacteria and protozoa	
Bacteria (*Vibrio fischeri)*	0.92 (EC50-5 min); 0.61 (EC50-15 min)
Ciliate (*Tetrahymena pyriformis)*	0.15 (LC50-24 h); 0.72 (LC50-48 h)
Plants	
Green algae (*Chlorella vulgaris)*	10 (LC50-96 h)
Green algae *(Selenastrum capricornutum)*	0.42 (LC50-96 h)
Invertebrates	
Water flea *(Daphnia magna)*	1.7 (LC50-24 h)
Shrimp (*Artemia salina)*	3.9 (LC50-24 h)
Vertebrates	
Bluegill sunfish (*Lepomis macrochirus)*	0.12 (LC50-24 h)
Channel catfish (*Ictalurus punctatus)*	0.068 (LC50-96 h)
Mouse (*Mus musculus)*	116 mg/Kg of body weight (oral LD50)
Rat (*Rattus norvegicus)*	50 mg/Kg of body weight (oral LD50)

EC50 is the effective concentration that causes 50% inhibiting effect.

LC50 is the lethal concentration that kills 50% of the test organisms.

LD50 is the lethal dosage per unit body weight that kills 50% of test organisms.

Table 1.4 The criteria for rating active ingredient of pesticides under the USEPA acute toxicity rankings system (Ma *et al.*, 2002b).

USEPA categories and warning labels			**Acute toxicity to rats**
Category	PAN Narrative Rating*	Warning	Oral LD50 (mg/Kg)
1	Highly toxic	Danger	< 50
2	Moderately toxic	Warning	50-500
3	Slightly toxic	Caution	500-5,000
4	Not acutely toxic	None	> 5,000

*PAN denoted Pesticide Action Network

Table 1.5 List of chemicals evaluated for carcinogenic potential maintained by USEPA Office of Pesticide Programmes (OPP) in 1996 (Ma *et al.*, 2002a).

	Category	Description
A	Known to cause cancer in human	Sufficient epidemiological data to support the carcinogenicity of the substance
B	Probable human carcinogen	Known to cause cancer to animals but not definitively in human
	B1: Sufficient evidence from animal studies but limited evidence in humans	
	B2: Sufficient evidence from animal studies but inadequate or no data in humans	
C	Possible human carcinogen	Limited evidence of carcinogenicity in the absence of human data
D	Not classifiable as to human carcinogenicity	Incomplete data, but further research is needed
E	Probably not carcinogenic	No evidence in at least two adequate animal tests in different species

chlorines *para* to the hydroxyl group increases the toxicity, since it has a lower steric hindrance to transport across the cell membrane and bring into effect (Ruckdeschel *et al.*, 1987; Litchfield & Rao, 1998; Fong, 2001). Therefore, some degradates of PCP may show higher toxic effect but some would not, depending on different positions and number of chlorine on them. After all, PCP exerts high toxic effect to human as well as the other organisms, no matter it can be degraded or not.

1.1.5 Remediation of pentachlorophenol

As described in the previous section, PCP is recognized as a highly toxic compound. Owing to this reason, PCP has been ranked into the U.S. List of Priority Pollutants for Remediation Treatment (Chapman *et al.*, 1982; Chiu *et al.*, 1998; Pohland *et al.*, 1998). Thus, the remediation of PCP has been of great interest recently (Chiu *et al.*, 1998; Litchfield & Rao, 1998; Jiahlong *et al.*, 2000). The conventional strategies for remediation of aqueous PCP contaminations involve physical, chemical and biological treatment, which are introduced in the following sections.

1.1.5.1 Physical treatment

Physical treatment involving air stripping or activated carbon is recommended by USEPA to decontaminate drinking water (Vidal, 1998). However, these technologies are being objected. One of the reasons is that PCP is not readily vaporized (as mentioned previously); air stripping is not very efficient. Meanwhile, adsorption on activated carbon is efficient as it can remove high concentration of PCP (Nelson & Yang, 1995; Mollah & Robinson, 1996a; Hu *et al.*, 1998; Slaney & Bhamidimarri, 1998). One hundred % of PCP (with initial 100 mg/L) can be removed by granular activated carbon column after 24 h (Mollah & Robinson, 1996a). However, it is expensive, and PCP can only be recovered by applying very high temperature, which increases the cost and deteriorates the pore of activated carbon. As a result, it cannot be used repeatedly for adsorption and this further increases the cost (Danis *et al.*, 1998). On the contrary, both technologies merely transfer the problem from one medium (water) to another (air or activated carbon), but without thoroughly treating PCP (Chiu *et al.*, 1998; Vidal, 1998; Fong, 2001).

1.1.5.2 Chemical treatment

A number of treatments involving chemical processes can be applied to remediate PCP. Firstly, incineration or pyrolysis over 300°C can be employed (Environmental Health Criteria, 1987). It can destroy the compound in a short time. However, they are expensive and yield the higher toxic compound, PCDDs and PCDFs (mentioned in Section 1.1.3) as a result. Therefore, ozonation is introduced. Ozonation is a powerful degradation process that involves ozone (O_3), a strong oxidant, to oxidize PCP. However, it is unstable in solution, which causes the process not readily reproducible. And extra caution should be taken as it easily causes explosion (Bruchet *et al.*, 1992; Tseng & Huang, 1992).

Thus, another alternative, photocatalytic process, is a recent interesting route in remediating PCP in water, which can decontaminate high concentration of PCP at the same time (Bolduc & Anderson, 1997). To perform photocatalytic oxidation, it requires a photocatalyst initiated with light ($\lambda < 380$ nm) to produce a strong oxidizing agent ($\bullet OH$ or $\bullet O_2^-$). The photocatalyst has no change in structure and thus it can be reused. Usually, complete mineralization can be achieved to thoroughly solve the problem of pollutants (Bolduc & Anderson, 1997). However, it is not cost-effective if it is employed to treat low concentration of contaminant. Usually, pre-concentration can be done before applying for photocatalytic oxidation (Bolduc & Anderson, 1997).

1.1.5.3 Biological treatment

Bioremediation offers a feasible method for clean-up organic pollutants (Ragini, 1997). It is a general description of two processes, biodegradation and biosorption. The application of biodegradation usually involves together with biosorption, as the pollutant is firstly immobilized for degradation (Pignatello *et al.*, 1983). The decomposers, bacteria and fungi are employed in bioremediation process, as they possess diverse kinds of enzymes to degrade organic matter (Challon, 1997; Cauntú *et al.*, 2000). PCP biodegradation by different kinds of fungi and bacteria, as well as their degradation pathways have been well established (Laine & Jørgensen, 1996; Challon, 1997; Litchfield & Rao, 1998; Pohland *et al.*, 1998; Cauntú *et al.*, 2000). Table 1.6 lists some species which can be employed in biodegradation of PCP. Virtually, the pathway of PCP degradation depends on the

Table 1.6 Examples of microbial species capable of degrading PCP.

Class	Species	References
Bacteria	*Arthrobacter* sp.	Litchfield & Rao, 1998
	Bacillus subtilis	Litchfield & Rao, 1998
	Elodea canadensis	Pignatello *et al.*, 1983
	Flavobacterium sp.	Lewandowski & DeFilippi, 1998
	Mycobacterium chlorophenolicum	Brandt *et al.*, 1997
	Phanaerochaete chrysosporium	Cauntú *et al.*, 2000
	Potamogeton crispus	Pignatello *et al.*, 1983
	Pseudomonas cepacia	Lewandowski & DeFilippi, 1998
	Vibrio succinogenes	Lewandowski & DeFilippi, 1998
Fungi	*Phanerochaete chrysosporium*	Challon, 1997; Chiu *et al.*, 1998
	Phanerochaete sordida	Lewandowski & DeFilippi, 1998
	Phoma glomerate	Lewandowski & DeFilippi, 1998
	Saccharomyces cerevisiae	Lewandowski & DeFilippi, 1998
	Trichoderma sp.	Lewandowski & DeFilippi, 1998
Others	Activated sludge biomass	Pohland *et al.*, 1998; Jianlong *et al.*, 2000
	Spent mushroom substrates	Chiu *et al.*, 1998
	Spent compost from mushroom farm	Laine & Jørgensen, 1996

species and enzyme involved (Chiu *et al.*, 1998; Litchfield & Rao, 1998; Pohland *et al.*, 1998; Cauntú *et al.*, 2000). For example the PCP degradation by a species of nonwhite rot basidiomycete, *Mycena avenacea* generates intermediates tetrachlorobenzoquinone, (TeBQ), tetrachlorohydroquinone (TeHQ) and trichloro-hyrdrobenzoquinone (THBQ) (Kremer *et al.*, 1992) (Figure 1.3). But the PCP degradation metabolites from *Pseudomonas* sp. include 2,3,4,6-tetrachlorophenol, 2,3,5,6-tetrachlorophenol, and 2,3,5-trichlorophenol (Watanabe, 1973). These microorganisms can use PCP as sole carbon and energy source (Chu & Kirsch, 1972). And extracellular enzymes such as lignin peroxidase, or cell-bound enzymatic systems from the cells can attribute to degradation (Litchfield and Pao, 1998). However, the process is often too slow to be acceptable (more than 25 days) (Pignatello *et al.*, 1983; Mollah & Robinson, 1996b; Pohland *et al.*, 1998). In addition, it leads to transformation rather than mineralization of contaminants (Laine & Jørgensen, 1996); thus some highly toxic metabolites could be formed to increase the environmental problem.

Biosorption of organic pollutants has received increasing attentions in recent years (Jianlong *et al.*, 2000; Schiewer & Volesky, 2000). It is applied to prevent further spreading of pollutants, or to filter or pre-concentrate the pollutant for further treatment (Jianlong *et al.*, 2000). The biosorbents, the biological matters for pollutants adsorption, can be either viable or non-viable algae, bacteria, fungi and yeasts and their biopolymers, including chitin and chitosan, and even the biowaste such as crustacean shells (Knorr, 1991; Laine & Jørgensen, 1996; Brandt *et al.*, 1997; Challon, 1997; Jianlong *et al.*, 2000; No & Meyers, 2000). They can be obtained from natural environment, cultivation or processing waste industries. In addition, the pollutants can easily be recovered by simply changing pH (DiVincenzo & Sparks, 1997) or replacing the fresh PCP-free solutions, e.g. NaOH solution (Knorr, 1991; You & Liu, 1996; Brandt *et al.*, 1997; DiVincenzo & Sparks, 1997). Thus the biosorbents can be utilized again and further reduce the cost of treatment process. Therefore, comparing with activated carbon, biosorption is more economic and practical alternative. And thus biosorbents for remediation have been highly concerned and recommended rather than activated carbon, as the cost and availability are the criteria for choosing an adsorbent to remove organic pollutants (Viraraghavan & Slough, 1999). Meanwhile, biosorption is solely the process of phase transfer of the pollutant; further treatment should be employed to completely decontaminate the

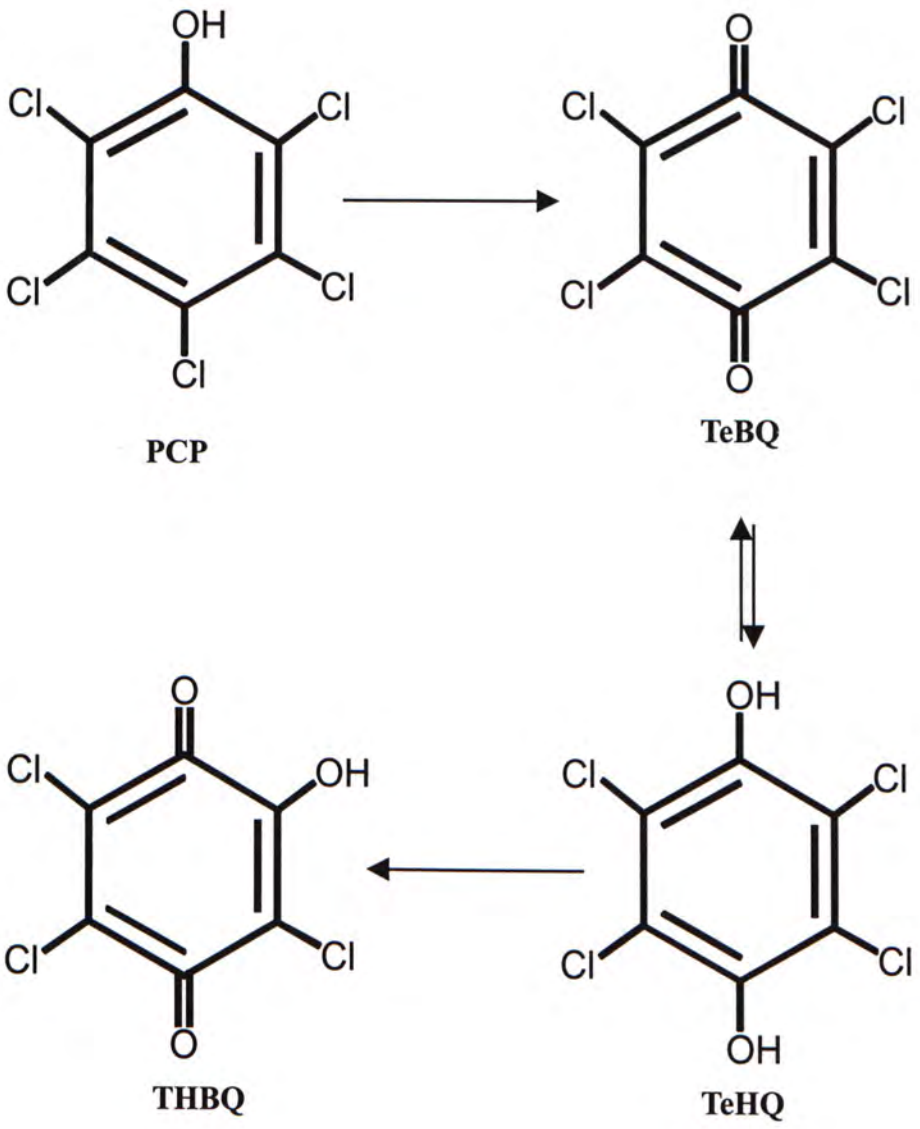


Figure 1.3 The biodegradation pathway of pentachlorophenol by the nonwhite rot basidiomycete, *Mycena avenacea* (Kremer *et al.*, 1992).

pollutants.

1.1.5.4 Alternative for combining two treatments

With reference of the above treatment methods, none of them perfectly fulfills under the consideration of economy and efficiency. Therefore, the application of two steps method seems to advance the efficiency of remediation (Mollah & Robinson, 1996a; No and Meyers, 2000). The processes of biosorption and photocatalytic oxidation (PCO) were combined in treating the target pollutant, PCP, in the present study. Biosorption is applied to pre-concentrate PCP or collect PCP from accidental spillage by cheap biosorbents; whereas, PCO is employed to treat the adsorbed PCP completely. This two-step approach can maximize the benefit from the point of economic of biosorption and the degree of efficiency of PCO (No & Meyers, 2000). To degrade the adsorbed PCP by PCO, it can be achieved in two approaches. The first is to desorb the PCP from the biosorbents. The eluent is then transferred for PCO. And the second approach is to treat the adsorbed PCP directly on the biosorbents. The latter approach has additional advantage with less treatment steps if the second approach is proven to be feasible. In the following sections, the details of biosorption and PCO employed in my study are introduced.

1.2 Biosorption

Biosorption, by definition, means the passive (i.e. not metabolically mediated) transport mechanisms starting with the diffusion of the target compound (PCP in this case) to the cellular compounds of biological species, bisorbent (Shumate & Strandberg, 1985; Challon, 1997; Schiewer & Volesky, 2000). Biosorption is generally used for the treatment of heavy metal pollutants in wastewater (Onsoyen & Skaugrud, 1990; Synowiecki & Al-Khateeb, 1997; Lau, 2000; No & Meyers, 2000; Sag & Kutsal, 2000; Tsui, 2000; An *et al.*, 2001). Meanwhile, application of biosorption for organic and other pollutants has also received increasing attentions in recent years (Brandt *et al.*, 1997; Muir, 1999; No & Meyers, 2000). It has been shown in recent studies that biomass has the ability to concentrate the organic compounds, such as PCP as those hydrophobic organic pollutants show a high tendency to accumulate into the biomass (Laine & Jørgensen, 1996; Brandt *et al.*, 1997; Challon, 1997; Thome & Jeuniaux, 1997; Chiu *et al.*,

1998; Viraraghavan & Slough, 1999; Jiahlong *et al.*, 2000). Therefore, the biosorption can be applied to serve as the first step in wastewater treatment; that is to separate the hazardous pollutants.

In the real situation for PCP remediation, biosorption can be employed as industrial effluent treatment to remove PCP from discharge; or employed as an emergency tool to prevent the dispersal of accidental spillage. Biosorption is selected as it has advantages over the others. Firstly, most biosorbents show high efficiency in removing many kinds of pollutants including PCP in rapid rate, especially at low concentration (1-100 mg/L) (Jacobsen *et al.*, 1996; Brandt *et al.*, 1997). Secondly, biosorption is cost-effective process as the PCP adsorbed on biosorbents can be recovered by changing pH or replacing the fresh solution as mentioned in Sections 1.1.5.3 (Knorr, 1991; Brandt *et al.*, 1997). Therefore the biosorbent can be reused to lower the operation cost. In addition, the cost of biosorbents is low, compared with that of activated carbon. This can be accounted from the sources of biosorbents. The living or dead biosorbents can be obtained directly from the abundant biological materials found in nature or its by-products, or cultivation from large-scale fermentation industries. Furthermore, the biosorbent is non-toxic and biodegradable, which can be easily disposed either by biodegradation or incineration without further treatment (Olsen *et al.*, 1996; Hirano *et al.*, 2000; Wu *et al.*, 2000). Thus, biosorption can be regarded as an effective and economical technology for removal PCP.

A variety of biomass can be employed as biosorbents for PCP. Table 1.7 shows some examples of biomass which can be employed for PCP biosorption. Chitinous materials have been proven to be cost effective and good biosorbents for heavy metals (Knorr, 1991; Chui *et al.*, 1995; Rülcker *et al.*, 1995; Kawamura *et al.*, 1997; Tsui, 2000; Wu *et al.*, 2000). But few studies investigate their ability for removal PCP, or compare the abilities among chitinous materials. Only modified chitosan was utilized to remove PCP by Thome & Jeuniaux (1997) (see Section 1.2.1.5). In my study, chitinous materials, crude chitin, pure chitin and chitosan were employed to test the feasibility to remove PCP. The information of chitinous materials is given as following.

Table 1.7 Examples of biosorbents for PCP.

Class	Species	Reference
Bacteria	*Mycobacterium chlorophenolicum*	Brandt *et al.*, 1997
Fungi	*Caenorhabditis elegans*	Challon, 1997
	Rhizopus arrhizus	Challon, 1997
Others	Activated sludge biomass	Jiahlong *et al.*, 2000
	Anaerobic acidogenic systems	Piringer & Bhattacharya, 1999
	Anaerobic granular sludge	Kennedy *et al.*, 1992; Tham & Kennedy, 1994; Kennedy & Pham, 1995
	Chitinous materials	Thome & Jeuniaux, 1997
	Peat-bentonite mixtures	Viraraghavan & Slough, 1999
	Spent oyster mushroom substrate*	Chiu *et al.*, 1998
	Straw compost from mushroom farm*	Laine & Jørgensen, 1996

* The biomass is also responsible for biodegradation.

1.2.1 Chitin and chitosan

1.2.1.1 History of chitin and chitosan

Chitin was firstly described by Braconnot in 1811 who was a professor of Natural History, Director of the Botanical Garden and member of the Academy of Sciences of Nancy, France (Muzzarelli, 1977). He isolated an alkali-resistant fraction from a mushroom, *Agaricus volvaceus* and other mushrooms with diluted warm alkali. Then he named this fraction as "fungine". After a series of experiments, he discovered this "fungine" was quite unique among the plants and it seemed to contain more nitrogen than wood (Muzzarelli, 1977; Knorr, 1991; George & Martin, 2000; Tsui, 2000). After 12 years, Odier discovered an insoluble substance from insect exoskeleton (Muzzarelli, 1977). He called this substance as "chitin", and he suggested that it was the basic material of exoskeletons of all insects. The chitin was not well recognized until 1878 that Ledderhose clearly established the hydrolysis equation of chitin from glucosamine and acetic acid (Muzzarelli, 1977; Tsui, 2000).

Chitosan was discovered by Royget in 1859 once he boiled chitin in a very concentrated potassium hydroxide solution (George & Martin, 2000). He noticed that this substance became soluble in organic acids and turned violet by diluted iodine and acid solution. Then he called this substance as modified chitin. And this modified chitin was named as chitosan by Hoppe-Seyler in 1894 (Muzzarelli, 1977; George & Martin, 2000; Tsui, 2000). After that a great deal of fundamental studies took place on chitin and chitosan. An intense interest in new applications grew in the 1930s and early 1940s (George & Martin, 2000).

1.2.1.2 Structures of chitin and chitosan

Chitin is the second most common polysaccharide in the world after cellulose (Onsøyen & Skaugrud, 1990). It is a long and unbranched water-insoluble polysaccharide (Olsen *et al.*, 1996; Ravindra *et al.*, 1998; George & Martin, 2000). Its structure differs from cellulose only by replacing an acetyl amide group (-$NHCOCH_3$) to the hydroxyl group (-OH) on the second carbon of each sugar sub-unit, making chitin a polymer with 2000 – 4000 units of 2-acetamido-2-deoxy-D-glucopyranose (Shiau and Yu, 1998; Savant and Torres, 2000) (Figure 1.4a).

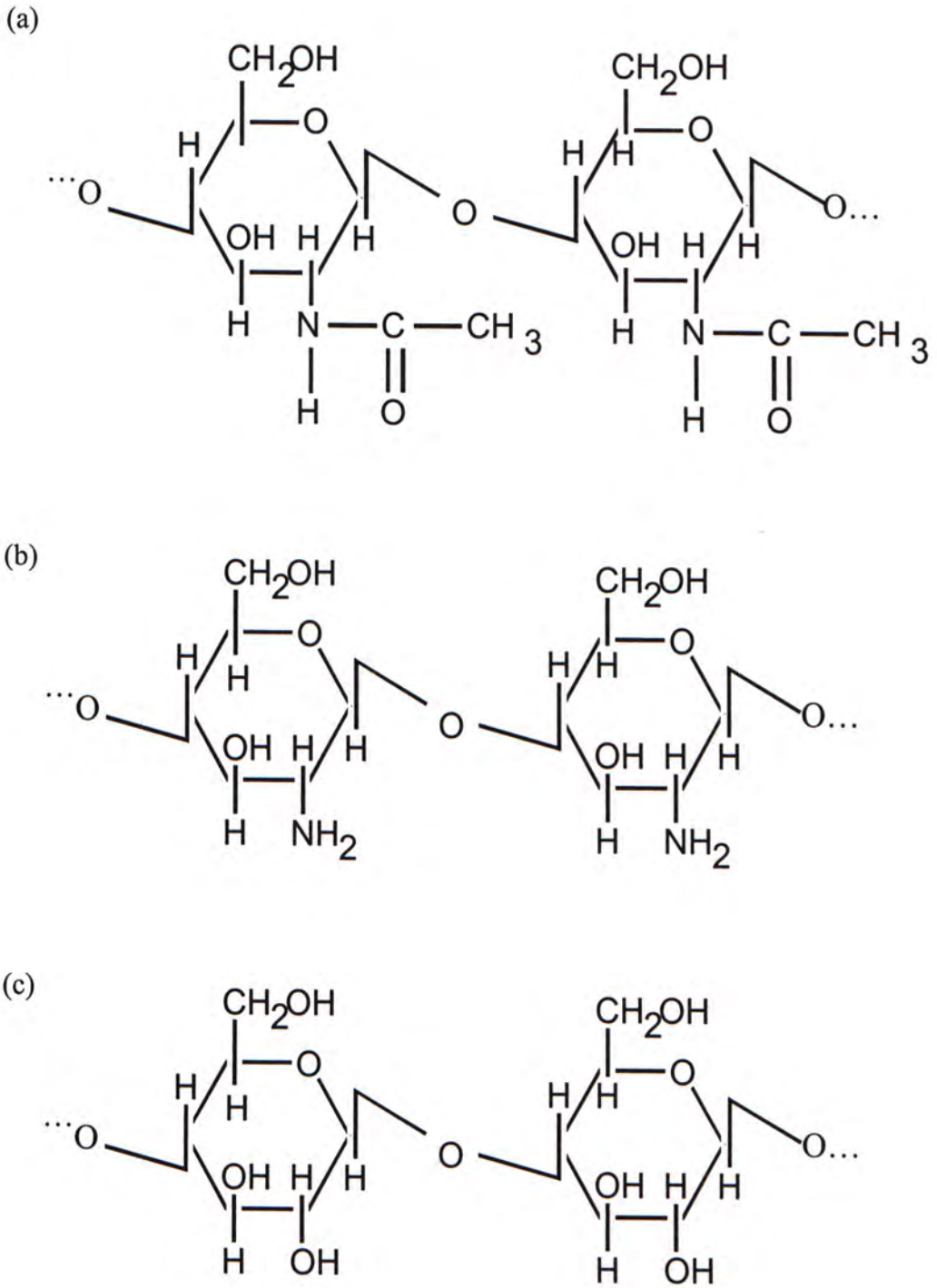


Figure 1.4 Chemical structures of (a) chitin, (b) chitosan and (c) cellulose (Goosen, 1997; Shiau and Yu, 1998; Savant & Torres, 2000).

These units are linked by β(1→4) glucosidic bonds (Hirano *et al.*, 1999).

The amide group is fairly reactive which allows chitin to be easily derivatized. By removing the acetyl functional group (-$COCH_3$) from the amino groups (-NH-), the chitosan, the major derivative of chitin with monomer 2-amino-2-deoxy-D-glucopyranose (Figure 1.4b), can be obtained (Hirano *et al.*, 2000). Owing to the presence of a primary amine group (-NH_2), chitosan becomes positively charge in acidic water solution (Olsen *et al.*, 1996; Savant & Torres, 2000). This property makes it readily dissolve in various acidic solvents forming viscous non-Newtonian solutions (Olsen *et al.*, 1996; George & Martin, 2000). It is worth to note that chitin itself can have some deacetylation, but as a rule of thumb the material is only called chitosan after more than 60-70% of the acetyl groups are removed (Goosen, 1997).

1.2.1.3 Sources of chitin and chitosan

Chitin is the major structural component of the crustacean exoskeleton like krill, crab, shrimp and lobster (Onsøyen & Skaugrud, 1990). In addition, it is also the natural biopolymer abundant in the fungal cell wall (White *et al.*, 1979; George & Martin, 2000; Savant & Torres, 2000). Therefore, chitin can be obtained from seafood processing waste of crustacean, mainly from shrimp, crab and shellfish, or from cultivation of fungi. Table 1.8 shows the relative amounts of chitin in shellfish and the mycelium of various fungi. However, production of chitin from food processing waste has advantage over the fungi by relieving the burden of total solid waste. It is estimated that there is 50-90% of total solid waste landings brought from shellfish processing (Savant & Torres, 2000).

To obtain chitin and chitosan from the crustacean shells, the extraction process was followed as shown in Figure 1.5. Virtually, the extraction processes can be employed with various chemicals and conditions. However, different extraction processes directly affect the physico-chemical properties of extracted chitin and chitosan. Therefore, it is necessary to develop a standardized extraction method.

Crustacean shell waste consists of protein (20-40%), calcium and magnesium carbonate (30-60%), chitin (20-30%) and lipids (0-14%) varying with species and season (Onsøyen & Skaugrud, 1990; Hirano *et al.*, 1999; Goycoolea *et*

Table 1.8 Relative amounts of chitin in shellfish and the mycelium of various fungi (modified from Knorr, 1991; George & Martin, 2000; Goycoolea *et al.*, 2000)

Product	**Type/species**	**Chitin content (%)**
Crustacean	Crab	14-30
	Shrimp	13-30
Fungi	*Mucor rouxii*	9.4
	Tremella mesenterica	3.7
	Penicillium chrysogenum	8.0-11.9
	Paracoccidioides brasiliensis	11
	Saccharomycapsis gutulata	2.3
	Blastomyces dermatitidis	13

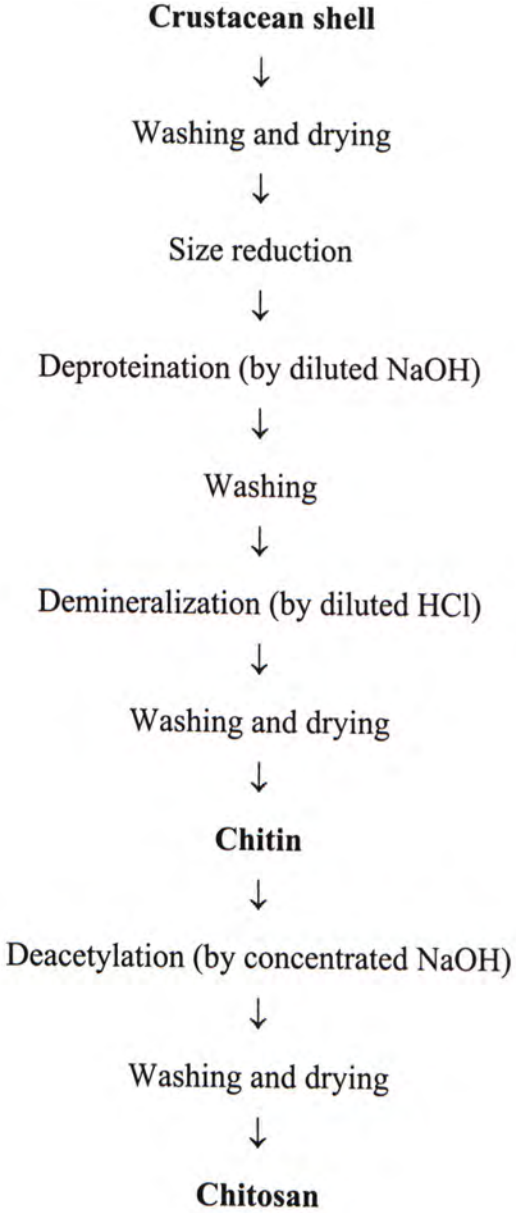


Figure 1.5 Flow diagram of general chitin and chitosan processing (modified from Onsøyen & Skaugrud, 1990; Knorr, 1991).

al., 2000). Therefore, the isolation of chitin from crustacean biowaste should aim at removing the residual protein and inorganic matter. Firstly, the crustacean shell is washed to remove any attach residue (Figure 1.5). In this step, only 10% of solid waste is produced in the case of manufacture crude chitin from shrimp shell (*Penaeus japonicus*) (reported from ICPI). Then chitin can be obtained from the processes of deproteination and demineralization after drying and reducing size. In most conventional chemical treatments, the process of deproteination involves using 0.25-2.5 M of NaOH at temperature 65-100°C for 0.5-72 h; whereas using HCl with concentration higher than 1.4 N at room temperature for demineralization (Rane & Hoover, 1993; Goycoolea *et al.*, 2000). For the production of chitosan, the chitin is deacetylated by concentrated NaOH (50%) at 100°C for 4 h (Daele & Thomé, 1986; George & Martin, 2000).

It is noteworthy that up to 70% and 80% of crustacean biomass remains as waste after the production of chitin and chitosan respectively (George & Martin, 2000). Therefore, there is recent interest in using crustacean shell for wastewater treatment instead of extracted chitin and chitosan, as the shell is more economic with less treatment steps and waste products (Chui *et al.*, 1996; An *et al.*, 2001). Unfortunately, there are seldom studies comparing the ability of the crustacean shell, chitin and chitosan. It gives additional advantage to the use of crustacean shell if the toxicants removal ability of it is higher than or similar as that of chitin and chitosan.

1.2.1.4 Application of chitin and chitosan

Due to the unique structures of chitin and chitosan, they possess a wide range of properties, such as high amine content (Rane & Hoover, 1993; Muzzarelli & deVincenzi, 1997; Koide, 1998), biodegradable and biocompatible (Rane & Hoover, 1993), non-toxic (Chui *et al.*, 1996; Olsen *et al.*, 1996) and capability of emulsification (George & Martin, 2000). Furthermore, chitosan has additional properties like high solubility, polycationic nature, and possessing antimicrobial properties (Rane & Hoover, 1993), coagulating property (Rane & Hoover, 1993; Lee & Jo, 1998; Shimojoh *et al.*, 1998; George & Martin, 2000), hemostatic and antithrombogenic functions (Chui *et al.*, 1996; Hirano *et al.*, 2000). Owing to these unique properties, they have received increased attention for its commercial applications such as pharmaceutical use, wastewater treatment, biotechnology and food and beverages, etc. (Table 1.9).

Table 1.9 Applications of chitin and chitosan.

Chitinous materials	Application areas	References
Chitosan	**Pharmaceutical use**	
	- Lowering cholesterol	Trautwein *et al.*, 1997
	- Wound healing dressing	Ikejima & Inoue, 2000; Hirano *et al.*, 2000
	Wastewater treatment	
	- Heavy metal e.g. Cu(II), Pb(II) and Hg(II)	No & Meyers, 2000; Dantas *et al.*, 2001
	- Dairy waste	Olsen *et al.*, 1996
	- Dye	Synowiecki & Al-Khateeb, 1997
	- PCB	No & Meyers, 2000 Knorr, 1991
	- PCP	Thome & Jeuniaux, 1997
	Biotechnology	
	- Cell immobilization	Rane & Hoover, 1993
	- Membrane filtration	Savant & Torres, 2000
Chitin	**Food and beverages**	
	- Conversion to single cell protein	Cosio *et al.*, 1982
	- Clarification of juices	Synowiecki & Al-Khateeb, 1997
	Wastewater treatment	
	- Heavy metal e.g. Cu(II), Pb(II) and Hg(II)	George & Martin, 2000
	- PCB	No & Meyers, 2000

Among all, the current major applications are in industrial wastewater treatment (Ganjidoust *et al.*, 1996; Synowiecki & Al-Khateeb, 1997; No & Meyers, 2000; Dantas *et al.*, 2001). Chitinous materials can effectively function as adsorbents in wastewater treatment to remove and recover the metal ions like Cu^{2+}, Pb^{2+} and Hg^{2+}, as well as organic compounds such as protein, dye and organochlorinated compounds (Daele & Thomé, 1986; Onsøyen & Skaugrud, 1990; Chui *et al.*, 1996; Synowiecki & Al-Khateeb, 1997; Yoshida & Takemori, 1997; Tsui, 2000; Wu *et al.*, 2000; Wu *et al.*, 2001). Utilization of chitinous materials on metal ions removal has been well studied recently (Rülcker *et al.*, 1995; Vasconcelos *et al.*, 1997; No & Meyers, 2000; Tsui, 2000). The presence of amino and hydroxyl groups of chitin and chitosan can form a coordination complex with metal ions. In addition, the mechanisms like ion-exchange, adsorption and chelation can also be attributed to remove metal ions (Onsøyen & Skaugrud, 1990; Knorr, 1991; Tsui, 2000). The metal ions removal by chitinous materials is remarkably efficient. Almost 100% removal efficiency is always achieved in many studies on removing ions of copper, iron (II), iron (III), mercury, nickel, zinc and cobalt, etc (Knorr, 1991; George & Martin, 2000; Tsui, 2000).

On the contrary, the purification of organic pollutants such as dye, protein (Knorr, 1991; Olsen *et al.*, 1996; Cho *et al.*, 1998; No & Meyers, 2000; Wu *et al.*, 2000) and some xenobiotics like PCB and PCP from contaminated water with chitinous materials were shown to be apparently effective, even better than activated charcoal (Knorr, 1991; Thome & Jeuniaux, 1997). However, the complicate mechanisms are still not clear.

1.2.1.5 Study on PCP removal by chitinous material

Thome & Jeuniaux (1997) studied the PCP sorption ability by a modified chitosan, gluteraldehyde cross-linked and $NaBH_3CN$ reduced chitosan (CHT-Glu-Red) (with granulation < 325 mesh). This modified chitosan was proved to be effective in removing organic compounds such as PCB. It appeared to have removal ability 10 times higher than the unmodified chitosan based on the preliminary results. The authors filtered 5 mg/L of PCP solutions through 1 g of the biosorbent. The results showed that 98% of PCP could be removed. On the contrary, more than 77% of PCP could be desorbed simply by a strong base (0.01 M of NaOH). Therefore, it was postulated that the mechanism of PCP sorption on the chitosan derivative was

probably the weak chemical interaction, for example, dipole-dipole attraction. Given that the weak forces were easily destroyed by the ionic interactions existing between a strong base (NaOH) and a weak acid (PCP). The authors suggested that the presence of amino group was requisite to provide the chitosan adsorptive properties for PCBs and PCP (Thome & Jeuniaux, 1997).

1.2.2 Factors affecting biosorption

The ability of biosorption depends on different physico-chemical conditions. Providing that the conditions is optimal, it yields higher removal efficiency. Therefore, to realize the factors affecting biosorption is necessary.

1.2.2.1 Solution pH

It is well documented that the pH of the solution is an important parameter which can largely influence the consequence of biosorption (Hu *et al.*, 1998). This effect is related to the change of various groups on surfaces of the biosorbents as well as the sorbates, the adsorbed molecules. As mentioned in Section 1.1.1, PCP is a hydrophobic ionizable organic compounds (HIOCs) with a pK_a of 4.74. With pH < 4.74, most PCP appears as protonated form (uncharged). This uncharged compounds behave similarly as other hydrophobic non-ionizable organic compounds (HNOCs) and readily adsorb on organic matter biosorbent (Viraraghavan & Slough, 1999; DiVincenzo & Sparks, 2001). On the other hand, in alkaline condition, the adsorption of ionized form (phenolate ion) may result in a more specific reaction with the functional groups on the surface of sorbents. In addition, the adsorbability of ionized and neutral forms of PCP can be also estimated by the logarithm of octanol-water partition coefficient, log P_{ow}, which is the ratio of a neutral compound in octanol and water phases (Equation 1.3).

$$P_{ow} = ([HA]_o + [A^-]_o) / ([HA]_w + [A^-]_w) \qquad (1.3)$$

where the subscripts o and w refer to octanol and water phases, respectively; whereas [HA] and [A^-] are the concentrations of the uncharged and ionic species of the pesticide respectively. P_{ow} is a measure of hydrophobicity of xenobiotics, PCP (Toro & Horzempa, 1982; Smejtek & Wang, 1993; Gremaud & Turesky, 1997; Danis *et al.*, 1998; Hu *et al.*, 1998).

It is reported that log P_{ow}(HA) of PCP is 5.1 and log P_{ow}(A) is 1.5 (Leo *et al.*, 1981; Smejtek & Wang, 1993; Danis *et al.*, 1998). That is, the hydrophobicity of neutral form is approximately five times more than that of ionized form. It indicates that the neutral form, appearing at low pH, is readily adsorbed on organic sorbents such as activated carbon and chitinous materials. This explanation is reasonable matched with the results of many studies that adsorption increases with the decrease of pH (Jianlong *et al.*, 2000; DiVincenzo & Sparks, 2001).

Meanwhile, changing pH also results from altering the characteristics of the biosorbents, chitinous materials. It makes the interaction of PCP and chitosan more complicated. By considering chitosan first, the intrinsic pK_a of its amine functions is close to 6.5 (Kjøniksen *et al.*, 1997; Piron *et al.*, 1997). That is it exhibits as polycationic in water at pH < 6.5. In neutral or alkaline conditions, chitosan loses its charge (Kjøniksen *et al.*, 1997; Piron *et al.*, 1997). Therefore, the mechanisms of PCP adsorption on chitosan changing with pH is intricate. This might involve the proportion of neutral or ionized PCP and that of chitosan at definite pH. For the biosorption on chitin, it is also dependent on changing pH. It might be also attributed to the protonation and deprotonation of the amino groups of the *N*-acetyl-D-glucosamine units present on chitin. However, the behavior and mechanisms of biosorption are still unclear (Brandt *et al.*, 1997). And the optimal pH solution is only found by balancing effect.

Moreover, it is observed that the pH of solution changes after the addition of chitinous materials. Therefore, even the optimal solution pH has been reached, the biosorption might not seem ideal. The differences between the initial pH and the final pH of the solution play an important role to determine the biosorption ability (Ning *et al.*, 1999; Jianlong *et al.*, 2000). Thus, the application of buffer solution with optimal pH range might enhance the biosorption, though it can increase the cost of operation.

1.2.2.2 Concentration of biosorbent

The effect of biosorption is highlighted by the results of varying biosorbent concentration. Generally, the biosorbent concentration increases, the total amount of sorbate (PCP) adsorbed on biosorbent (removal efficiency, RE) increases. But conversely, the amount of PCP adsorbed on unit dry mass of biosorbent (removal capacity, RC) decreases (O'Connor & Connolly, 1980; DiVincenzo & Sparks, 1997;

Ning *et al.*, 1999; Jianlong *et al.*, 2000). The decrease in removal capacity was due to binding sites of the biosorbent remaining unsaturated during the adsorption reaction. On the other hand, if the biosorbent concentration is low, sorbate molecules are forced to occupy the limited amount of biosorbents, which leads to the increase of RC. But the overall PCP removal decreased (Ning *et al.*, 1999).

1.2.2.3 Retention time

The process of biosorption is known to be time dependent, since the time is required for sorbate (PCP) to diffuse in the solution medium and form physico-chemical bonding with the biosorbent. In general, the biosorption process is a biphasic mechanism. The first step is intraparticle diffusion, i.e. the surface diffusion (Slaney & Bhamidimarri, 1998; Guibal *et al.*, 1999). It is the initial rapid step which contributes significantly to the uptake of sorbate, as the sorbents as well as the sorbate are sufficiency available (Tsui, 2000). DiVincenzo & Sparks (1997) reported that this rapid initial stage results in 68% of the PCP being sorbed on soil at the first 20 min. The second step is the interparticle diffusion, which is the mechanisms combined of mass transport into the biosorbent particles (Slaney & Bhamidimarri, 1998) and intraparticle diffusion under the extremely low solute concentrations left in the solution (Wu *et al.*, 2001). It requires longer time for the sorbates to diffuse and adsorb on the deep central of biosorbents. DiVincenzo & Sparks (1997) described an additional 10% of PCP was then sorbed on 2 days. This is so-called the true equilibrium time. The equilibrium is defined by USEPA that the minimum amount of time is needed to establish a change of less than 5% of solute concentration (Viraraghavan & Slough, 1999).

Previous studies indicted that the biosorption mechanisms reflected that the actual equilibrium time usually prolonged to a few hours to a day (DiVincenzo & Sparks, 1997; Guibal *et al.*, 1999; Viraraghavan & Slough, 1999; Jianlong *et al.*, 2000). The equilibrium time for the adsorption of ammonium molybdate by chitosan performed by Guibal *et al.* (1999) was found to be 20 h. Viraraghavan & Slough (1999) reported that the removal of PCP from water by peat-bentonite mixture dropped rapidly within the first hour and subsequently decreased more gradually until 8 h, reaching the actual equilibrium time. However, in the real situation of bioremediation, it is impossible to accept this long-time process to merely obtain the slight increase of removal. Therefore, the biosorption is usually taken within one

hour, as the intraparticle diffusion is the rate limiting mechanism (Viraraghavan & Slough, 1999).

1.2.2.4 Temperature

The efficiency of the sorbate-sorbent system is dependent on the factor of temperature (Mehrian *et al.*, 1991; Király & Dékéány, 1996; Mobed & Chang, 1998; Sag & Kutsal, 2000; DiVincenzo & Sparks, 2001). Firstly, high temperature can increase the kinetic energy of the sorbates as well as the sorbents and thus increase the rate of adsorption. In addition, temperature can have effects on solubility and ionization constants (Mobed & Chang, 1998; DiVincenzo & Sparks, 2001). Mehrian *et al.* (1991) reported that the protonated species of PCP increased with increasing temperature. Therefore, increasing temperature can enhance the adsorption theoretically, if only the factor of ionization constant is taken into account. However, it is required to consider the temperature effect on the bonding formation between the sorbent and sorbate (Mehrian *et al.*, 1991; Mobed & Chang, 1998; DiVincenzo & Sparks, 2001). It is suggested that if the adsorption of protonated species increases with temperature (entropy-driven process), hydrophobic bonding is said to be attributed to the adsorption mechanisms (Mehrian *et al.*, 1991; Mobed & Chang, 1998). On the other hand, if the adsorption of ionized species declines with the increase of temperature, it indicates that the adsorption process is exothermic (Mehrian *et al.*, 1991; Király & Dékéány, 1996; DiVincenzo & Sparks, 2001). This suggests that the adsorption is due to the reaction of a specific surface functional group, such as the formation of hydrogen bonding and charge transfer (DiVincenzo & Sparks, 2001). Meanwhile, the increase of temperature can also be considered to enhance the desorption process; and thus decrease the amount of sorbate present on sorbent (Mobed & Chang, 1998).

1.2.2.5 Agitation rate

It is easy to realize that the increase of agitation rate gives better contact between of sorbent and sorbate and hence advance the adsorption rate. Therefore, the experiments are usually performed with shaking (Nelosn & Yang, 1995, Brandt *et al.*, 1997; DiVincenzo & Sparks, 1997; Jianlong *et al.*, 2000; An *et al.*, 2001; DiVincenzo & Sparks, 2001). However, one additional factor needs to be considered is the economic point, as the increase of the shaking rate raises the operational cost.

It is requisite to take the benefit between these two factors.

1.2.2.6 Initial sorbate concentration

The adsorption of organic compounds is obviously influenced by the initial concentrations of such organic compounds based on many literatures (O'Connor & Connolly, 1980; DiVincenzo & Sparks, 1997; Guibal *et al.*, 1999). Firstly, the sorbate concentration takes part in influencing the sorption rate in batch systems. The transfer rate surrounding the sorbents is directly proportional to the sorbate concentration. This is because diffusion is in part controlled by a concentration gradient, higher concentration exhibits higher gradient and hence faster diffusion (O'Connor & Connolly, 1980; DiVincenzo & Sparks, 1997; Guibal *et al.*, 1999). Meanwhile, under the low concentration, the sorption rate is lower as for the less favorable concentration gradient and also the blockage the entrance of the sorbate pores (DiVincenzo & Sparks, 1997; Guibal *et al.*, 1999).

Secondly, the increase of initial sorbate concentration results in increasing RC. This is due to a higher probability of contact between sorbates and sorbents (Jianlong *et al.*, 2000). Generally, RC increases steeply at low sorbate concentrations and eventually reached a plateau as the sorbate concentrations increased (DiVincenzo & Sparks, 1997; Tsui, 2000). That is, the influence is primarily significant for low concentrations (Guibal *et al.*, 1999). However, biosorption involving different biosorbents as well as sorbates may show different equilibrium isotherm. Therefore, two widely accepted and easily linearized monolayer adsorption models, Langmuir and Freundlich adsorption isotherms are usually adopted to characterize the adsorption (You & Liu, 1996; Jianlong *et al.*, 2000). The models are described in following sections.

1.2.3 Modeling of biosorption

The biosorption model is requisite to compare the capability and selectivity among different biosorbents, which is derived from the adsorption isotherm. The adsorption isotherm is performed by varying the initial sorbate concentrations under the constant temperature. Two commonly used monolayer adsorption models, Langmuir and Freundlich adsorption isotherms are usually employed (Mollah & Robinson, 1996a; You & Liu, 1996; Aksu, 1998; Ning *et al.*, 1999; Tsui, 2000; An *et al.*, 2001).

1.2.3.1 Langmuir adsorption model

The Langmuir isotherm can be derived by assuming that only monolayer adsorption on a surface containing a finite number of identical sites. And it also assumes uniform energies of adsorption over the surface of adsorbents (so-called homogeneous), no transmigration of adsorbate in the plane of the surface, and no interaction between the adsorbed molecules (Scott & Karanjkar, 1995; Mollah & Robinson, 1996a; You & Liu, 1996). The Langmuir model can be expressed as

$$q_e = b\, C_e\, q_{max} / (1 + b\, C_e) \qquad (1.4)$$

where q_e is the amount of solute adsorbed per unit dry weight of biosorbent at concentration C_e (mg of PCP/g of biosorbent); C_e is the equilibrium concentration of solute in bulk aqueous phase after adsorption (mg/L); q_{max} represents the theoretical maximum uptake of solute per unit dry weight of biosorbent (mg of PCP/g of biosorbent); b denotes the adsorption affinity constant related to energy of adsorption (L/mg). In order to find out q_{max} and b easily, the Langmuir equation is transformed into linear form as shown below.

$$\frac{C_e}{q_e} = \frac{1}{bq_{max}} + \frac{C_e}{q_{max}} \qquad (1.5)$$

or

$$\frac{1}{q_e} = \frac{1}{bq_{max}} + \frac{1}{bC_e\, q_{max}} \qquad (1.6)$$

q_{max} and b can be found from the slope and y-intercept of a plot of C_e/q_e against C_e (or $1/q_e$ against $1/C_e$) respectively. Thus these adsorption constants can be used to compare the adsorption behavior in different adsorbate-adsorbent systems (Viraraghavan & Slough, 1999).

1.2.3.2 Freundlich adsorption model

The Freundlich model has been widely adopted to characterize a single-solute adsorption of organic pollutants from water (Scott & Karanjkar, 1995; Mollah & Robinson, 1996a; Brandt *et al.*, 1997; Jianlong *et al.*, 2000). The applicability of

empirical Freundlich equation is considered valid for heterogeneous surface character of adsorbents. By means of the adsorbents are expected to have heterogeneous energies for adsorbing adsorbates. Therefore the energy varies as a function of the surface coverage, and as a result of variation in the heat of sorption (Scott & Karanjkar, 1995; Viraraghavan & Slough, 1999; Jianlong *et al.*, 2000; Tsui, 2000). The equation has the generally form as shown below.

$$q_e = k\, C_e^{1/n} \qquad (1.7)$$

where q_e is the amount of solute adsorbed per unit dry weight of biosorbent at concentration C_e (mg of PCP/g of biosorbent); C_e represents the equilibrium concentration of solute in bulk aqueous phase after adsorption (mg/L); k and n are empirical constants which k is the adsorption capacity and n is the adsorption intensity.

The empirical constants can be determined easily from the plot of linearized logarithmic equation (Scott & Karanjkar, 1995; Viraraghavan & Slough, 1999; Jianlong *et al.*, 2000).

$$\text{In } q_e = \text{In } k + 1/n \text{ In } C_e \qquad (1.8)$$

To plot a straight line of In q_e against In C_e, k and n can be read from the slope and y-intercept. The magnitude of n indicates the system suitability, with values of n>1 representing favorable adsorption conditions; whereas k is related to the capacity of adsorbent for the adsorbate (Scott & Karanjkar, 1995; Tsui 2000).

1.3 Photocatalytic degradation

Photocatalytic oxidation (PCO) is a class of heterogeneous advanced oxidation processes (AOPs) in which semiconductor oxides are utilized as catalysts to largely increase the reaction rate and efficiency (Jardim *et al.*, 1997). AOPs is a series of physico-chemical processes which an energy source (e.g. ultraviolet, visible light or sunlight) is employed to generate the strong oxidizing agents (hydroxyl radical •OH and superoxide radical $\bullet O_2^-$ anion). These oxidizing agents can quickly oxidize the organic compounds.

PCO is of great interest for the elimination of hazardous organic wastes even in the μg/L or low mg/L range (Matthews, 1987; Crittenden *et al.*, 1996; Ma *et al.*, 2001). It is a low-temperature nonenergy-intensive approach for chemical waste destruction (Ma *et al.*, 2001). As a result, it can enhance the efficiency of bioremediation. A wide range of organic compounds has been reported to be effectively degraded via heterogeneous photo-oxidation on different semiconductor oxides, including haloalkanes and haloalkenes, aromatics, toluene, benzene, atrazine and dye (Fong, 2001) (Table 1.10).

1.3.1 Titanium dioxide

Among the various semiconductor oxides used in photocatalysts e.g. titanium dioxide (TiO_2), zinc oxide (ZnO) and cadmium sulphide (CdS), TiO_2 has been investigated in more detail during the past decade (Jardim *et al.*, 1996; Ma *et al.*, 2001). The application of TiO_2 materials in photocatalytic processes was firstly introduced in the 1970s (Fujishima & Honda, 1972; Ma *et al*, 2001). But it was widely used only after 1983 that organic pollutant oxidative mineralization enhanced by TiO_2 was clearly recognized for the first time (Pruder & Ollis, 1983; Ma *et al*, 2001). It has been proven to be the prominent semiconductor for effective decomposition of organic pollutants in air and water (Jardim *et al.*, 1996; Yu *et al.*, 2000; Pecchi *et al.*, 2001). The properties of TiO_2 are shown in Table 1.11. TiO_2 has remarkably performance because the anatase TiO_2 has suitable thermodynamic position of the valence and conduction bands (Yu *et al.*, 2000). In addition, it is nontoxic and stable to resist to photocorrosion and chemical corrosion (Zhang *et al.*, 1998; Yu *et al.*, 2000; Ma *et al.*, 2001).

1.3.2 Mechanism of photocatalytic oxidation using photocatalyst TiO_2

The mechanism of PCO using TiO_2 is presented by schematic diagram in Figure 1.6 (Matthews, 1986; Turchi & Ollis, 1990; Crittenden *et al.*, 1996; Yu *et al.*, 2000). TiO_2 is illuminated by light rays in ultraviolet region with wavelength below 380 nm, electron in valence band is excited across the bandgap to conduction band, leaving holes behind in the valence band, and results in forming the hole and electron

Table 1.10 Organic pollutants that can be degraded by photocatalytic oxidation (PCO) (modified from Fong, 2001)

ORGANIC POLLUTANTS	REFERENCES
Haloalkanes and haloalkenes	
Chloroform ($CHCl_3$)	Chandrasekharaiah *et al.*, 1994; Ollis *et al.*, 1995
Trichloroethylene ($CHCl=CCl_2$)	Tseng & Huang, 1992
Aromatics	
Phenol	Riyad & Ali, 1999
Chlorophenol	Ollis *et al.*, 1995
e.g. Pentachlorophenol (PCP)	Mill & Hoffmann, 1993; Pecchi *et al.*, 2001
2,3,5-trichlorophenol (2,3,5-TCP)	Jardim *et al.*, 1997
2,4-dichlorophenol (2,4-DCP)	Riyad & Ali, 1999
Polychlorinated biphenols (PCBs)	Ollis *et al.*, 1995
e.g. 2-chlorobiphenyl (2-CB)	Bush *et al.*, 1998
3,3'-dichlorobiphenyl (3,3'-DCB)	Hong & Wang, 1999
Dioxins	Chandrasekharaiah *et al.*, 1994
e.g. 2,7-dichlorodibenzo-p-dioxin (2,7-DCDD)	Ollis *et al.*, 1995
Polychlorinated dibenzonfurans (DCDF)	Hentunen *et al.*, 2000
Polyaromatic hydrocarbons (PAH)	Augugliaro *et al.*, 2001
Toluene	Fujihira *et al.*, 1981
Benzene	Fujihira *et al.*, 1981
Atrazine	Ollis *et al.*, 1995
e.g. 2-chloro-4-ethylamino-6-isopropylamino-s-triazine	Augugliaro *et al.*, 2001
Dyes	
Acid Blue 9 (AB-9)	Yang *et al.*, 2001
Remazol Black B	Arslan *et al.*, 2000
Reactive red	Duran *et al.*, 2000

Table 1.11 Characteristics of titanium dioxide.

Characteristics		**References**
Band gap energy (eV)	3.2	Zhang *et al.*, 1998; Baudin *et al.*, 2000
Absorption spectrum (nm)	< 380	Baudin *et al.*, 2000
Particle size (nm)	20-30	Arslan *et al.*, 2000
Isoelectric point	6.8	Zhang *et al.*, 1998; Pecchi *et al.*, 2001
Crystalline phases	Anatase, brookite and rutile	Yu *et al.*, 2000; Pecchi *et al.*, 2001

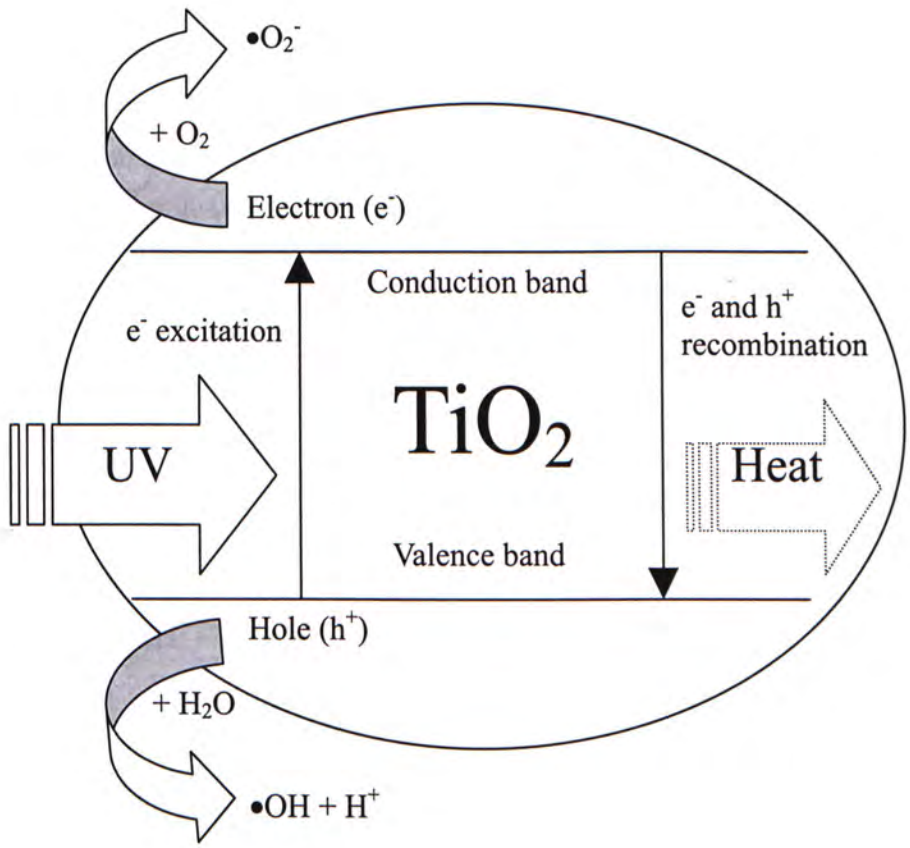


Figure 1.6 The schematic diagram of initial mechanism of photocatalytic oxidation with TiO_2 (Matthews, 1986; Turchi & Ollis, 1990; Crittenden *et al.*, 1996; Yu *et al.*, 2000).

pairs (h^+ and e^-) (Equation 1.9). The electron can then react with oxygen molecules (Equations 1.12 and 1.14); whereas the hole reacts with water molecules or hydroxide ions (OH^-) (Equations 1.10 and 1.11). Under the series reactions, the strong oxidizing agent (•OH) is generated (Equations 1.10 and 1.13). The production of hydroxyl radicals (•OH), a short-lived and extremely potent oxidizing agent, can initiate PCO on TiO_2 surface or diffuse into bulk solution (Jardim *et al.*, 1996). With the addition of hydrogen peroxide (H_2O_2), more •OH are generated as expressed in Equations 1.15, 1.16 and 1.17. Therefore, H_2O_2 is usually added for enhancement of the degradation efficiency of PCO. In addition, oxygen can be supplied as electron acceptor to prolong the recombination of electron-hole pairs during PCO (Vidal, 1998; Yu *et al.*, 2000).

$$TiO_2 + h\nu \rightarrow h^+ + e^- \quad (1.9)$$

$$h^+ + OH^- \rightarrow \bullet OH \quad (1.10)$$

$$h^+ + H_2O \rightarrow OH^- + H^+ \quad (1.11)$$

$$e^- + O_2 \rightarrow \bullet O_2^- \quad (1.12)$$

$$2\bullet O_2^- + 2H_2O \rightarrow 2OH^- + 2\bullet OH + O_2 \quad (1.13)$$

$$2e^- + O_2 + 2H^+ \rightarrow H_2O_2 \quad (1.14)$$

$$H_2O_2 + e^- \rightarrow \bullet OH + OH^- \quad (1.15)$$

$$H_2O_2 + \bullet O_2^- \rightarrow \bullet OH + OH^- + O_2 \quad (1.16)$$

$$H_2O_2 + h\nu \rightarrow 2\bullet OH \quad (1.17)$$

By considering the degradation process of organic compounds, there are two proposed photocatalytic mechanisms. One suggests that the oxidation of organic compounds is firstly initiated by the free radicals in the aqueous solution. The other proposal states that the organic compound has to be firstly adsorbed on the catalyst surface and then reacts with hydroxyl radicals from adsorbed water to form the final products (Yang *et al.*, 2001). However, recent studies (Vidal, 1997; Pecchi *et al.*, 2001; Yang *et al.*, 2001) apparently support the latter proposal. Therefore, the surface area and charge of the catalysts may play an important role in the catalytic process. Given that the larger the area of catalyst, the more reactant can adsorb on surface for reaction (Pecchi *et al.*, 2001). On the contrary, the complementary charge of catalyst and reactant can favour the adsorption and hence increase in the activity.

The point of zero charge (pzc) of TiO_2 is at pH pzc = 6.8. That is, the surface is positively charged under acidic condition (pH<6.8) whereas it is negatively charged in alkaline media (pH>6.8) (Zhang *et al.*, 1998; Yu *et al.*, 2000; Pecchi *et al.*, 2001). Therefore, the adjustment of pH leads to vary the surface charge of TiO_2 and hence influences the reaction ability (Yu *et al.*, 2000).

1.3.3 Advantages of photocatalytic oxidation with TiO_2 and H_2O_2

PCO is a non-selective powerful degradation process with stable and reproducible performance. It can degrade a wide range and a high concentration of organic pollutants within a short processing time (Fong, 2001). Above all, PCO can lead to complete mineralization (i.e., oxidation to CO_2 and H_2O) of a variety of hydrocarbons, (Zhang *et al.,* 1998; Yu *et al.*, 2000; Pecchi *et al.*, 2001) which can thoroughly abolish the pollutants, but not merely transfer the pollutants from one place to another.

As mentioned before, TiO_2 is a good photocatalyst which can largely enhance the reaction due to its suitable thermodynamic position of the valence and conduction bands. In addition, it aggregates to form cluster in micron range in aqueous solution by Van der Waals forces (Geissen & Xi, 2001). Therefore, it can be easily separated from the treated sewage discharge by membrane filtration, or simply by overnight sedimentation. And this collected TiO_2 can be used again by feeding into the reactor due to its stable property. On the contrary, TiO_2 is non-toxic, which makes the process more economic and environmental friendly.

With the addition of H_2O_2, the degradation efficiency can be much more advanced owing to the increase production of oxidizing agent, hydroxyl radical (•OH). Furthermore, it is relatively non-toxic and freely soluble in water (Hager, 1990). The addition of H_2O_2 in PCO process is highly recommended.

1.3.4 Degradation of PCP by photocatalytic oxidation

Only a few studies have investigated the degradation of aqueous PCP by PCO (Mills & Hoffmann, 1993; Jardim *et al.*, 1997; Fong, 2001, Pecchi *et al.*, 2001). In these experiments, pure PCP is irradiated by UV light (254 nm < λ < 380 nm) with TiO_2 and oxygen supply. The transient species of PCP were identified by either HPLC or GCMS and were summarized in Table 1.12. And the proposed degradation

Table 1.12 The intermediates of PCP formed during PCO.

Intermediates	**Irradiation time for 100% degradation**	**Reference**
- TeHQ; - ρ-chloroanil; - o-chloroanil; - acetate; and - formate.	3 h	Mills & Hoffmann, 1993
- 2,3,5,6-TeHQ; - 2,3,5,6-TeBQ; and - 2,3,5,6-TeCP.	6 h	Jardim *et al.*, 1996
- 2,3,5,6-TeHQ; - 2,3,5,6-TeBQ; - 2-penten-2-ol; and - pentanal.	1 h	Fong, 2001

TeHQ stands for tetrachlorohydroquinone; chloroanil is tetrachlorobenzoquinone; TeCP is tetrachlorophenol; TeBQ is tetrachlorobenzoquinone.

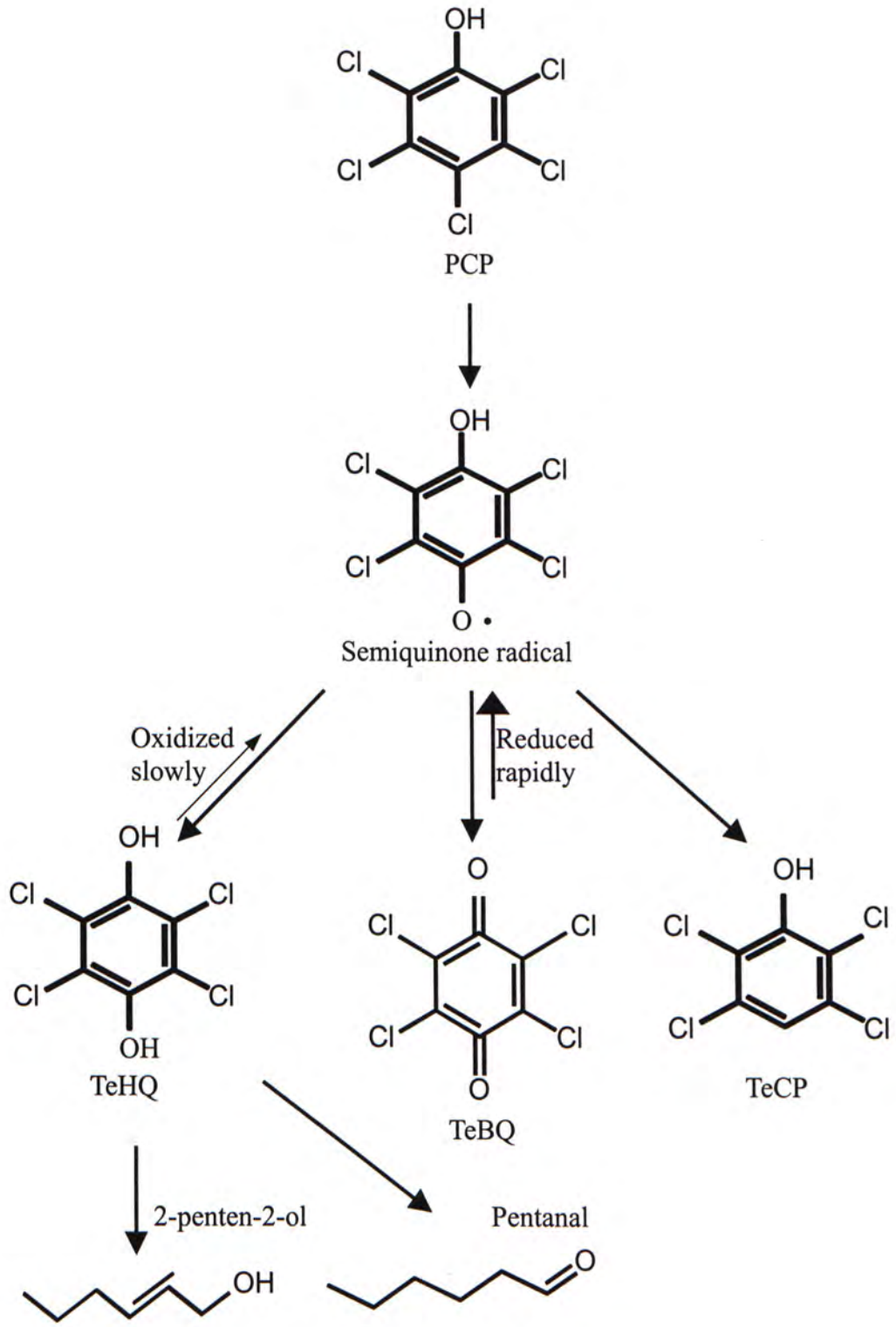


Figure 1.7 The proposed degradation pathway of PCP by photocatalytic oxidation (derived from Mills & Hoffmann, 1993; Jardim *et al.*, 1997; Fong, 2001).

pathway of PCP by PCO was demonstrated in Figure 1.7 (derived from Mills & Hoffmann, 1993; Jardim *et al.*, 1997; Fong, 2001). It was postulated that •OH preferred to attack on the *para* position of the PCP aromatic ring first as for the orientation tendency. Thus a semiquinone radical was formed and then proceed the oxidation and reduction to give TeHQ and TeBQ (Mills & Hoffmann, 1993; Fong, 2001). Whereas, TeBQ can be reduced into semiquinone radical rapidly, while TeHQ can be oxidized slowly (Mills & Hoffmann, 1993). Thus, the formation of TeHQ was dominant over TeBQ. Thereafter, ring cleavage was happened to form 2-penten-2-ol and pentanal (Fong, 2001). And the complete mineralization was proved to be achieved with the reference of monitoring the total organic carbon (TOC) (Jardim *et al.*, 1997) and chloride ions concentration (Mills & Hoffmann, 1993). In addition, all studies claimed that the reaction was taken on the surface of TiO_2 in which PCP was adsorbed. Therefore, the PCO rate was influenced by the solution pH as this could determine the adsorption of PCP on TiO_2 (Pecchi *et al.*, 2001) as mentioned in Section 1.3.2.

Jardim *et al.* (1997) discovered that the irradiated solutions were more toxic to *Escherichia coli* than the solution containing the starting compound, PCP. However totally different results were obtained by Fong (2001) who suggested that PCO could reduce the toxicity of PCP solution towards *Vibrio fisheri* (Microtox® test) and *Hyale crassicornis* (amphipod survival test). This might be the result of the sensitivity of different testing organisms as well as the experimental conditions. Meanwhile, there are few studies investigating the organic compound degraded on the sorbents directly by PCO. Therefore, in my study, the feasibility of PCO for this semi-liquid phase of PCP was tested.

2. Objectives

In order to reduce the environmental pollution caused by pentachlorophenol (PCP), a technology combined with two effective methods, biosorption and photocatalytic oxidation (PCO), is employed in this study. Biosorption is utilized to immobilize the pollutant and PCO is applied to completely destroy the pollutant.

In the part of biosorption, the chitinous materials, crude chitin, pure chitin and chitosan were utilized as biosorbents. The objective of the present study was to determine the effects of physico-chemical parameters in removing PCP, including solution pH, concentration of biosorbents, retention time, temperature, agitation rate and initial PCP concentration. The PCP removal efficiency (RE) and removal capacity (RC) of chitinous materials were found, and the two monolayer adsorption models, Langmuir and Freundlich isotherms were employed to determine the suitability and capacity of sorbate-sorbent systems. After that, a promising biosorbent with the highest efficiency among three potential candidates was screened out and used for further treatment.

For PCO, the adsorbed PCP on the selected biosorbent was degraded in PCO reactor under the selected conditions. The effects of irradiation time, biosorbent concentration and initial PCP concentration on biosorbent were determined. Then the intermediates were identified by gas chromatography/mass spectrometry (GC/MS) and the toxicity of irradiated solutions was monitored by Microtox® test. In addition, the biosorbents after treated by PCO were collected and analyzed by chitin assay, protein assay, diffuse reflectance Fourier transform infra-red (DRFT-IR) spectroscopy and biosorption efficiency to see any change in structure and removal efficiency. Then the performance of multiple biosorption and PCO cycles from these PCO treated biosorbents was determined.

3. Materials and methods

3.1 Biosorbents

Three different biosorbents, chitin A (crude chitin), chitin B (pure chitin) and chitosan (deacetylated chitin) (Plate 3.1), were purchased from International Chitin Production Inc. (ICPI, Vancouver, Canada). They were obtained from the shells of pink shrimp (*Penaeus japonicus*) collected from shrimp meat canning industry with undergoing a serial of treatments.

3.1.1 Production of biosorbents

The procedures for production of biosorbents were performed by ICPI. Fresh shrimp shells were collected and washed under tap water (1:15 w/v) followed by deionized water (1:1 w/v) to remove any remaining attached flesh. The washed shells were collected by centrifugation at 300 rpm and then dried at 92°C by tumbling drying process for one and a half hour. Afterwards, the shells were ground down to 100 mesh (0.15 mm) at 25°C and the powder obtained was named as chitin A (Tsui, 2000).

To produce chitin B, chitin A was undergone demineralization with 0.5 N HCl (1:10 w/v) at 25°C for 8 h to remove the minerals such as calcium carbonate and calcium chloric acid (Knorr, 1991), then neutralized with deionized water (3:1 w/v) and drain-dried by 100 mesh rayon bag. Afterwards, the demineralized chitin was deproteinized by 0.5 N NaOH (1:10 w/v) at 25°C for 8 h, then washed with deionized water (3:1 w/v) and again drain-dried by 100 mesh rayon bag. The demineralized and deproteinized chitin was then collected by centrifugation at 300 rpm and dried at 92°C by tumbling drying process for one and a half hour. Finally, the chitin was ground down to 100 mesh at 25°C and the powder was called chitin B (Tsui, 2000).

Chitosan was prepared by deacetylation of chitin B with 10 N NaOH (1:10 w/v) at 105°C for 45 min in order to hydrolyze the N-acetyl-linkage (Knorr, 1991). The deacetylated chitin was then rinsed by deionized water and collected by centrifugation at 300 rpm. Finally, this so-called chitosan was dried at 92°C by tumbling drying process for one and a half hour and ground down to 100 mesh at 25°C (Tsui, 2000).

(a)

(b)

(c)

Plate 3.1. The appearance of (a) chitin A, (b) chitin B and (c) chitosan.

3.1.2 Scanning electron microscope of biosorbents

The biosorbents were observed under scanning electron microscope (SEM) to examine the pores and bulky structures. The freeze-dried biosorbents were coated with alloy of gold and platinum by a sputter coater for 15 minutes (Edwards S150B). After placing in a scanning electron microscope (JSM-5300, JOEL, Tokyo, Japan), images were captured by camera, and the SEM photos were shown in Plate 3.2.

3.1.3 Pretreatment of biosorbents

Biosorbents were pretreated before used by washing with Milli-Q ultrapure water (Millipore, Bedford, UK) in a ratio of 1:15 (w/v) for 1 hr at 200 rpm by an orbital shaker (Lab-line 4628-1, Melrose Park, USA) and then collected by centrifugation with a Beckman J2-M1 centrifuge machine (Beckman, Fullerton, USA) at 14,000 rpm and 4°C for 30 min. After that the washed biosorbents were lyophilized by freeze-dryer (Labconco, Kansas City, USA) at 0°C at a reduced pressure for 5 days. The freeze-dried biosorbents were stored in an auto drybox (Eureka AD-75B, Taipel, Taiwan) for later experiments.

3.2 Pentachlorophenol preparation

Pentachlorophenol, PCP was purchased from Sigma Chemical (St. Louis, USA). The appearance of PCP was shown in Plate 3.3. Stock solution of 1,000 mg/L and 10,000 mg/L was prepared by dissolving 0.1 g and 1 g of solid PCP into 100 mL of 0.05 M of sodium hydroxide solution (Riedel-de Haën, Seeize, Germany) respectively and kept in darkness at 4°C before use (Mollah *et al.*, 1996a; Brandt, 1997).

3.3 Batch biosorption experiment

All biosorption experiments were performed by using batch systems under constant conditions. Fifty mL of PCP solution was prepared by dilution of stock solution with ultra-pure water in a 125 mL glass conical flask. The weighed freeze-dried biosorbents were added into the solution and agitated by an orbital shaker running at 200 rpm at 23±2°C. In particular time interval, 2 mL of mixtures were pipetted out and filtered by a filtering syringe with glass microfibre filter, GF/C

(a)

(b)

(c)

Plate 3.2 The scanning electron microscope photos of (a) chitin A, (b) chitin B and (c) chitosan. Image was taken by scanning electron microscope in power 75. The freeze-dried biosorbents were coated with alloy of gold and platinum by a sputter coater for 15 min.

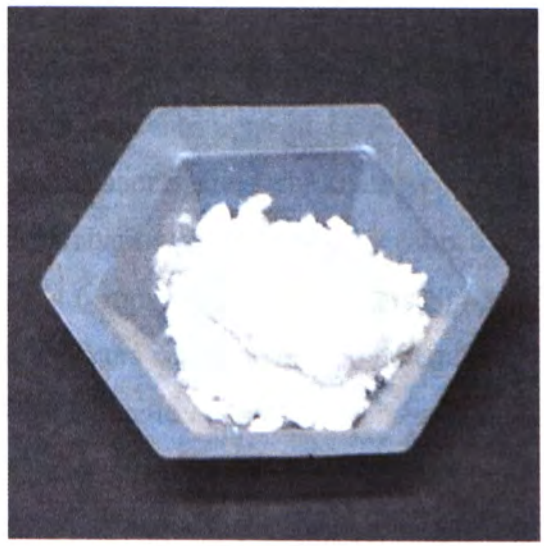


Plate 3.3 Appearance of pentachlorophenol (PCP).

(Whatman International Ltd., GF/C, Maidstone, England) (Hong & Wang, 2000). The final pH of mixtures was measured for each experiment. The concentration of PCP in filtrate was analyzed by reverse-phase high performance liquid chromatography (HPLC), which the conditions were mentioned in following section. In order to determine the amount of PCP retained by the container, control experiments were also performed under identical conditions, except that no biosorbent was added in the container. It was found that the loss of PCP in control was less than 1%. And it indicated extraneous PCP adsorption was negligible.

3.3.1 Quantification of pentachlorophenol by HPLC

The analytical concentrations of PCP in the filtrate were determined by reverse-phase high performance liquid chromatography (HPLC) (Chiu *et al.*, 1998; Hu *et al.*, 1998). The analysis was performed by using a Waters reverse-phase c18 (5 μm particle) column (4.6 mm x 250 mm, Waters Spherisob®, Waters Corporation, Milford, Ireland) on a Waters separations module equipped with a photodiode array detector (Waters 996). Isocratic elution was ran by mobile phase consisted of 75% acetonitrile, 24.875% distilled water, and 0.125% acetic acid at 1.5 mL min^{-1}. The maximum absorption wavelength was found to be 215 nm (modified from Chiu *et al.*, 1998). A series of PCP standards was prepared from 0.1 to 300 mg/L to construct a standard curve for quantification.

3.3.2 Data analysis for biosorption

For all the adsorption experiment, the PCP concentration analyzed by HPLC with the standard curve was transformed into removal efficiency (RE) and removal capacity (RC) by the following equations (Equations 3.1 and 3.2) (Nelson & Yang, 1995; Brandt *et al.*, 1997; Chiu *et al.*, 1998; Echeverria *et al.*, 1998; Guibal *et al.*, 1999), in order to compare the adsorption ability of three biosorbents.

$$RE\ (\%) = [(C_o - C_t) / C_o] \times 100\ \% \qquad (3.1)$$

$$RC\ (\text{mg of PCP/g of biosorbent}) = V(C_o - C_t) / W \qquad (3.2)$$

where RE is the percentage of PCP removed by the biosorbents (%), RC is the amount of PCP adsorbed per unit dry weight of biosorbent (mg of PCP/g of biosorbent), C_o is the initial concentration of PCP in solution (mg/L), C_t is the concentration of PCP in solution at time t (mg/L), W is the dry weight of biosorbent (g) and V is the volume of PCP solution (L).

Data in triplicate were analyzed statistically by one way ANOVA with $P<0.05$ followed by multiple comparison test (Tukey test) with a computer package of SigmaStat (Version 2.0, Jandel Scientific, Chicago, USA).

3.3.3 Selection of optimal conditions for batch PCP adsorption

In order to select the optimal conditions for PCP adsorption by the three biosorbents, the ability of biosorbents to adsorb PCP were determined under the constant condition with only one factor varying each time.

3.3.3.1 Effect of initial pH and biosorbent concentration

Effect of different biosorbent concentration in different initial pH of PCP solution was determined as following. A preliminary test was performed to determine the testing biosorbent concentration (0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 g/50 mL). As the deviation of RE and RC obtained from 0.025, 0.5 and 0.1 g biosorbent was too large, further refinement was considered justified; thus only 0.2, 0.4 and 0.8 g of biomass was considered. Ten mg/L of PCP solution was prepared by 100-fold dilution of 1,000 mg/L stock solution and the initial pH (2.5, 4.5, 6.5, 8.5 and 10.5) was adjusted by 1 M or 0.1 M of HCl (Univar, Seven-Hills, Australia) and NaOH (Riedel-de Haën, Seeize, Germany) respectively (Jacobsen *et al.*, 1996; Bissen *et al.*, 2001). The weighed biosorbent was added into 50 mL of pH adjusted PCP solution in glass conical flask shaken at 200 rpm at 23±2°C. Every 15 min interval, RE and RC of biosorbents for PCP adsorption were determined as described in Sections 3.3.1 and 3.3.2.

3.3.3.2 Improvement on pH effect and biosorbent concentration

Based on the results obtained from Section 3.3.3.1, pH 6.5 was chosen as the optimal biosorption condition. In order to kick out the change of pH in batches caused by addition of biosorbents, buffer solution was applied to maintain the pH at

constant 6.5 in order to determine the actual effect of batch pH. In this experiment, Tris buffer was utilized rather than phosphate buffer (Nelson and Yang, 1995; Jacobsen *et al.*, 1996), as the peak of phosphate in spectrum analyzed by HPLC interferenced with that of PCP. Tris buffer powder (Trizma® base), Tris [hydroxymethyl]aminomethane, was purchased from Sigma® Chemicals (St. Louis, USA). Stock solution of 0.8 M was prepared by dissolving the powder in a ratio of 1:10 (w/v) with ultrapure water. To obtain 10 mg/L of PCP with 50 mM of Tris buffer (Nelson & Yang, 1995) solution, 60 mL of Tris buffer stock solution was mixed with PCP solution to make the volume into 1 L with ultrapure water and the pH was adjusted to 6.5 by using HCl. Then the biosorption experiments were carried out as above.

3.3.3.3 Effect of temperature

Effect of temperature was investigated by placing batches with the optimal conditions determined in Section 3.3.3.1 which containing 50 mL of PCP solution (10 mg/L) with initial pH 6.5 and 0.4 g of biosorbent in orbital shaker shaken at 200 rpm and kept at constant temperature (22, 25, 30, 35 and 40°C). RE and RC of biosorbents at 60 min were determined as mentioned in Sections 3.3, 3.3.1 and 3.3.2.

3.3.3.4 Effect of agitation rate

Batch experiments as described in Section 3.3.3.2 were put in orbital shaker at different agitation rate (100, 200 and 300 rpm) with keeping the temperature at 25°C (the optimal temperature obtained from Section 3.3.3.2).

3.3.4 Effect of initial PCP concentration and biosorbent concentration

Batch experiments with the same conditions as described in Section 3.3.3.2 were applied except changing the biosorbent concentration into 0.2, 0.4 and 0.8 g, and the initial PCP concentrations into 5, 10, 100, 200 and 300 mg/L, prepared from the 1,000 and 10,000 mg/L of stock solution. The range of PCP concentrations were believed to cover most practical application for waste water treatment plant (Jacobsen *et al.*, 1996). Beyond this concentration was limited in this experiment due to the low solubility of PCP.

3.3.4.1 Adsorption isotherm

The adsorption data showing the effect of initial PCP concentrations and biosorbent concentrations obtained from Section 3.6.4 were analyzed with adsorption isotherms, Langmuir and Freundlich adsorption isotherms (Kawamura *et al.*, 1997). Two adsorption isotherms were described in Section 1.2.3.

3.4 Photocatalytic oxidation

For conducting the experiments of photocatalytic oxidation, PCO, the PCP adsorbed biosorbents, obtained from the process of optimal conditions biosorption determined in Section 3.3, were suspended in 100 mL of reaction mixture solution contained inside a Pyrex column (50 cm length x 2 cm internal diameter, 1mm thickness, 160-mL in volumn) and put inside the photocatalytic reactor. Inside the PCO reactor, air was pumped into the reaction mixture from the bottom of the column in order to distribute the biosorbents evenly in the solution. The reaction mixture solution for PCO consisted of 200 mg/L of titanium dioxide (TiO_2) and 6.7 mM of hydrogen peroxide (H_2O_2), which were the optimal conditions for photocatalytic oxidation of liquid phase PCP determined by Fong (2001). The detail of the reaction mixture solution was mentioned in following section.

3.4.1 Reaction mixture solution

Titanium dioxide (Degussa P25), the photocatalyst, was kindly provided by the Degussa Corporation (Frankfurt, Germany). It is a mixture of anatase and rutile TiO_2 as shown in Table 3.1. Ten thousand mg/L of TiO_2 stock solution was prepared by adding 1 g of TiO_2 powder into 100 mL of Milli-Q ultrapure water (Millipore, Bedford, UK).

Hydrogen peroxide (H_2O_2) was purchased from Riedel-de Haën (35% by weight, Seeize, Germany). The H_2O_2 stock solution (1 M) was made by 10 folds dilution with ultrapure water.

To prepare 100 mL of reaction mixture solution containing 200 mg/L of TiO_2 and 6.7 mM of H_2O_2, 2 mL of well shaken TiO_2 stock solution and 0.67 mL of H_2O_2 stock solution were pipetted into a 100 mL volumetric flask and the volume was made by adding ultrapure water.

3.4.2 Photocatalytic reactor

The photocatalytic reactor is composed of 8 ultraviolet (UV) lamps (15 watts, 365 nm, Vilber-Lourmat, Model T-15L/8D, Vernon Hills, USA) vertically surrounding the central Pyrex column to provide even irradiation on the reaction mixture (Plates 3.4b and 3.4c). The light intensity of the UV lamps was measured by a light meter (LI-250, LI-COR Inc., Lincoln, USA) and the readings were expressed in Table 3.2. The irradiation of the lamps were controlled by the control panel (Plate 3.4a). The reactor was covered with a stainless steel cylinder for protection from the potentially hazardous UV (Plate 3.4d). A fan was set on the top of the reactor to provide cooling effect during the reaction.

3.4.3 Batch photocatalytic oxidation system

The biosorbents obtained from the optimal conditions determined in Section 3.3 (0.4 g of chitin A in 10 mg/L or 100 mg/L PCP solution with initial pH 6.5 shaken at 200 rpm at room temperature for 60 min) were collected and separated with the PCP solution by suction filtration with glass microfibre filter, GF/C. The PCP concentration in filtrate was analyzed by HPLC as mentioned in Section 3.3.1. And hence the amount of PCP adsorbed on the biosorbents could be deduced. The collected biosorbents were totally transferred into 100 mL reaction mixture solution in a Pyrex column and irradiated by 8 UV lamps as described in Sections 3.4 and 3.4.1. To ensure the sufficient amount of biosorbents could be obtained for the later analysis, 50 mL of well-shaken reaction mixture was withdrawn each time. That was, the sample in each PCO experiment could be taken only twice. The sample was then filtered by suction filtration with GF/C filter paper. The filtrate was quantified by HPLC. And the collected biosorbents were undergone a process of extraction mentioned in later section so as to analyze the amount of PCP remained on the biosorbents. And thus, degradation efficiency (DE) and degradation capacity (DC) could be found which was expressed in later Section 3.4.5. In order to determine the amount of PCP lost during the transfer or uptake by the container, control experiments were also conducted along with all experiments under identical conditions, except that 100 mL of ultrapure water was utilized to replace the reaction mixture solution and no UV was irradiated.

Table 3.1 Composition of titanium dioxide (Degussa P25) (Sclafani, 1990).

Characteristics	Crystal form	
	Anatase	Rutile
Percentage by weight (%)	70-85	15-30
Surface area (m^2/g)	50-80	<10

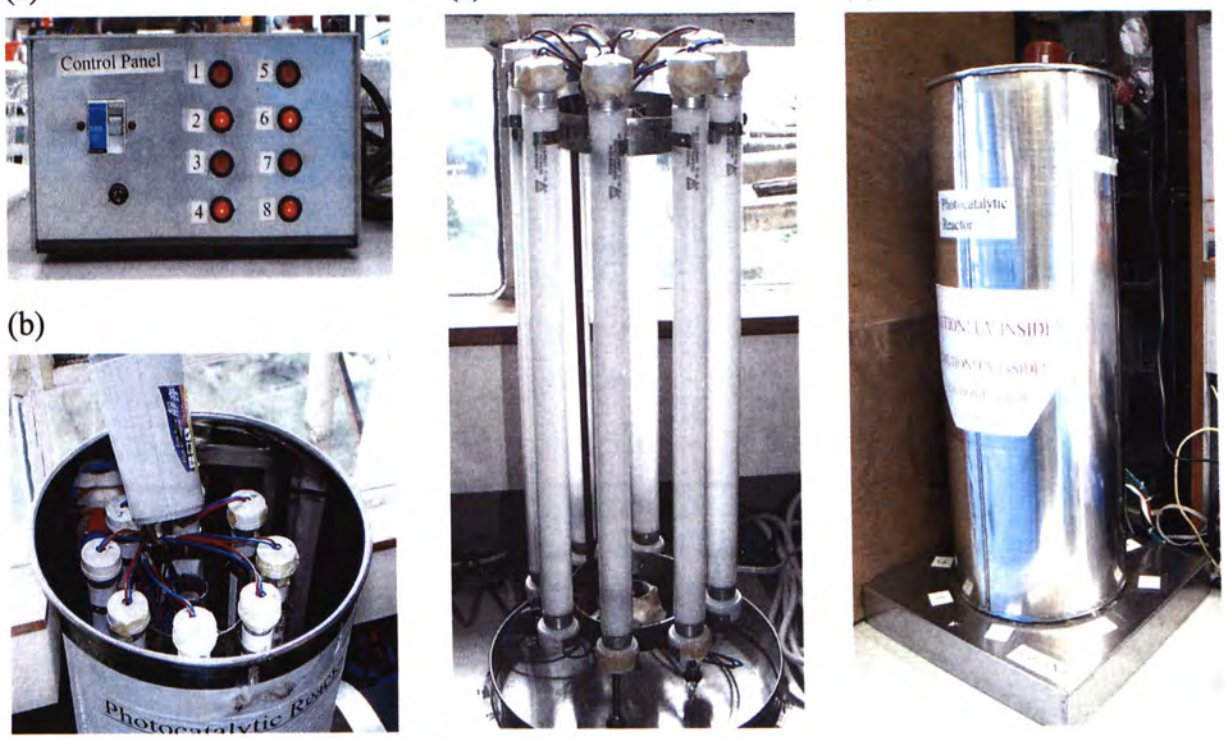


Plate 3.4 Control panel (a), top view (b), side view (c) and side view with stainless steel cylinder (d) of a photocatalytic reactor (From Fong, 2001).

Table 3.2 Light intensity of the UV lamps in the PCO reactor.

Lamp no.	**Light intensity (W/m^2)**
1	4.449
2	4.357
3	5.301
4	4.298
5	3.233
6	3.528
7	4.490
8	3.536
Average	4.149

3.4.4 Selection of extraction solvent

In order to determine the amount of PCP remained on the biosorbents, the biosorbents were extracted by the selected extraction solvent and then quantified by HPLC. To select a good extraction solvent, the biosorbents harvested from the optimal conditions batch determined in Section 3.3 (0.4 g chitin A in 50 mL of 100 mg/L of PCP solution with initial pH 6.5, shaken at 200 rpm at room temperature for 60 min) were collected by suction filtration with GF/C filter paper. The amount of PCP present in filtrate was determined by HPLC in order to find the amount of PCP adsorbed on the biosorbents. And the collected biosorbents were immersed into 50 mL of potential extraction solvents in 125 mL of conical flasks and returned to the orbital shaker at 200 rpm for 60 min. Based on preliminary results, the water-soluble solutions were chosen for the potential extraction solvents, which included 0.05, 0.5, 1, 5, 10 and 25 mM of NaOH (Mollah *et al.*, 1996a; Gremaud *et al.*, 1997; Matthews, 1987; Christov *et al.*, 1999) and 25, 50, 75 and 100% of methanol (Mallinckrodt ChromAR® HPLC grade, Paris, USA) (You & Liu, 1996; Gremaud & Turesky, 1997) diluted with ultrapure water. After 60 min extraction period, the solvents were separated from the biosorbents by suction filtration with GF/C filter paper and the amount of PCP was determined by HPLC. And thus the extraction efficiency could be found, which was expressed in Section 3.4.5. The solution with the highest extraction efficiency was selected as the extraction solvent for subsequent experiments.

3.4.5 Extraction efficiency

The extraction efficiency (EE) could be found by the equation below.

$$EE\ (\%) = 1 - [(A_o - A_b) / A_o] \times 100\ \% \qquad (3.3)$$

where EE is the percentage of PCP extracted from the biosorbents (%), A_o is the amount of PCP adsorbed on biosorbent (mg) and A_b is the amount of PCP extracted from biosorbent (mg).

Based on the results from the former section, Selection of extraction solvent, 75% of MeOH was utilized as the extraction solvent. For all the PCO experiments, the PCP from biosorbents was extracted by the method mentioned in Section 3.4.4 with 75% MeOH. To determine EE for each PCO experiment, the

biosorbents with the known PCP amount were undergone extraction with the same wet weight and treatment conditions as the samples.

3.4.6 Data analysis for PCO

For all the PCO experiments, the ability of the reaction was characterized with the degradation efficiency (DE) and degradation capacity (DC) shown in following equations.

$$DE\ (\%) = \{[A_o - A_s - A_b/EE]/ A_o\} x\ 100\ \% \quad (3.4)$$

$$DC\ (mg\ of\ PCP/g\ of\ biosorbent) = RC\ x\ DE \quad (3.5)$$

where DE is the percentage of PCP degraded by PCO (%), DC is the amount of PCP degraded per unit dry weight of biosorbent (mg of PCP/g of biosorbent), A_o is the initial amount of PCP on the biosorbents (mg), A_s is the amount of PCP in solution (mg), A_b is the amount of PCP remained on the biosorbents and TiO_2 in some cases (mg), EE is the extraction efficiency (%) and RC is the amount of PCP adsorbed on unit dry weight of biosorbent (mg of PCP/g of biosorbent).

All experiments were carried out in triplicate except Section 3.4.14, (Multiple biosorption and PCO cycles of PCO), which only duplicate was done, and no replicate was performed in Section 3.4.12 (Identification the intermediates of PCP degradation by PCO). Data were analyzed statistically by one way ANOVA followed by Tukey test ($P<0.05$).

3.4.7 Irradiation time

To establish the minimum time required for complete degradation of PCP, the performance of PCO were audited in the form of DE and DC against the irradiation time. From the preliminary results, the irradiation time was set as 0, 20, 40, 60, 120, 240, 360 and 480 min. Since each PCO batch could be withdrawn twice only as explained in Section 3.4.3, four independent batches should be prepared under the same conditions for conducting one complete irradiation time effect. In addition, the concentration of H_2O_2 was monitored along with the irradiation time by hydrogen peroxide cell test, stated in following section, to ensure the sufficient amount of H_2O_2 was available to produce hydroxyl radial for oxidation. According to

the results, H_2O_2 was spent over after four hours; therefore, additional 0.67 mL of H_2O_2 was added at 240 min irradiation.

3.4.8 Determination of hydrogen peroxide concentration

The concentration of H_2O_2 was determined by using the kit of hydrogen peroxide cell test (Spectroquant®, Merck, Darmstadt, Germany) following the procedures provided in the kit. In the presence of a solution acidified with sulfuric acid, hydrogen peroxide reacted with a titanic acid ester to form yellow peroxotitanic acids, for which the concentration of H_2O_2 could then be determined by a measurement of spectrophotometer (Milton Roy Spectronic 601, New York, USA) at 405 nm wavelength with a standard curve.

3.4.9 Effect of biosorbent concentration in PCO

In order to investigate whether the amount of biosorbents present for PCO would affect the PCO efficiency, the biosorbents from one, two and four independent biosorption batches (i.e. 0.4, 0.8 and 1.6 g of chitin A) prepared in optimal conditions (0.4 g of chitin A in 10 mg/L of PCP solution with initial pH 6.5 shaken at 200 rpm at room temperature) were collected respectively, and put for PCO reaction under the selected conditions with fixing the volume of reaction mixture solution in 100 mL as said in Sections 3.4.3 for 0, 20, 40, 60, 120, 240, 360 and 480 min. Additional 0.67 mL of 1M H_2O_2 was added at 240 min as mentioned in Section 3.4.7.

3.4.10 Effect of PCP amount on biosorbent in PCO

To evaluate the effect of initial PCP amount on biosorbents for PCO reaction, 0.4 and 0.8 g of biosorbents, prepared from the optimal conditions batches (based on the results in Section 3.3) in which the biosorbents were immersed in 10 mg/L and 100 mg/L of PCP solutions respectively, were utilized to conduct PCO reaction for 60 min. The irradiation time was set as 60 min based on the results in Section 3.4.9, as pronounced effect was expected.

3.4.11 Determination of chloride ion concentration and total organic carbon during PCO

To investigate the dechlorination degree of PCP by PCO, the concentration of free chloride ions in filtrate obtained in procedure of Section 3.4.9 after PCO were determined by an ion chromatographic system (Dionex DX500 Chromatography Systems, Dionex Corporation, Sunnyvale, USA) equipped with a conductivity detector (Dionex, model CD20), a gradient pump (Dionex, model GP40), a chromatographic enclosure (Dionex, model LC20) and an automatic sampler (Dionex model AS40). An ion exchange column (4 x 250 mm, AS4A-SC analytical column) was utilized with a mobile phase of carbonate-bicarbonate eluent prepared by 100-fold dilution of AS4A eluent concentrate (Dionex Corporation, Sunnyvale, USA), with ultrapure water at flow rate 2 mL/min (Mills and Hoffmann, 1993; Skurlatov *et al.*, 1997; Bissen *et al.*, 2001). The elution time of the chloride ion chromatographic peak output was 1.45±0.03 min. Three mL of filtrate at each irradiation time was brought to 2-fold dilution. And the chloride was quantified by measurement of peak area and determined from the standard curve prepared from 3, 6 and 12 mg/L of anion standard solution which were prepared by diluting 1, 2 and 4 mL of anion standards (Dionex Corporation, Sunnyvale, USA) to 10 mL with ultrapure water.

To determine the trend of total organic carbon (TOC) in filtrate, another 3 mL of filtrate was also diluted into two folds and analyzed by a total organic carbon analyzer (Shimadzu, model TOC-5000/5050, Kyoto, Japan) following the protocol of Instruction Manual (Shimadzu, Kyoto, Janpan) (Bolduc & Anderson, 1997; Feitz, 2000).

3.4.12 Identification the intermediates of PCP degradation by PCO

PCP degradation products after PCO were identified by gas chromatograph/mass spectrometry (GC/MS) with a gas chromatograph (Agilent, 6890 plus, Woodinville, England) equipped with a mass selective detector (Agilent, 5973N, Woodinville, England), an auto injector (Agilent, 7683, Woodinville, England) and a HP-5 MS column of 5% phenyl methyl silicone (30 m x 0.25 mm, 0.25 μm film thickness). The intermediates were identified by peak matching with the NIST98 MS library (Agilent, Woodinville, England) and pesticide MS spectral library (Agilent, Woodinville, England).

In order to harvest higher concentration of intermediates, 80 mL of filtrate after PCO (0.8 g of chitin A originally immersed in 100 mg/L of PCP biosorption batch and transferred to PCO reaction) was separately contained in four DCM rinsed 45-mL glass centrifuge bottles. Each was mixed with 10 mL of dichloromethane (DCM) (Mallinckrodt, ChromAR® HPLC grade, Paris, USA) after acidified by 0.5 mL of 1 M hydrochloric acid (DiVincenzo *et al.*, 1997; Skurlatov *et al.*, 1997). The sample was shaken for 2 h at 360 rpm in an incubator shaker (Edmund Bühler, Model TH15). Extracts were poured into a boiling tube and dried for 60 min by a nitrogen evaporator (Associates Inc., Organomation N-Evap, Berlin, USA) (modified from Gremaud and Turesky, 1996; Vidal, 1998). Pellet was resuspended by 0.5 mL of DCM and transferred into a micro-insert (Agilent, Woodinville, England) enclosed in 1.5-mL vial with screw cap (Agilent, Woodinville, England) after filtered by a syringe filter with 0.45 μm PTFE membrane (Acrodisc®CR, Ann Arobor, USA) with anhydrous sodium sulphate (Na_2SO_4) (AnalaR®, BDH, Poole, England) to absorb the left aqueous layer and stored at -20°C before GC/MS analysis (Skurlatov *et al.*, 1997). One μL of sample was injected in splitless mode for the analysis. The analytical conditions of GC/MS was listed in Table 3.3.

3.4.13 Evaluation of the change of PCO treated biosorbents

The biosorbents were required to evaluate with any changes after PCO reaction in order to establish whether they could be regenerated. To maximize the chance of PCO reaction with biosorbents, small amount of biosorbents, i.e. 0.4 g of chitin A, chitin B and chitosan were used and suspended into the reaction mixture solution mentioned in Section 3.4.1 for PCO with 60 min UV irradiation. Then these PCO treated biosorbents were filtered by GF/C filter paper and were further analyzed by chitin assay (Section 3.4.13.1), diffuse reflectance Fourier transform infra-red spectroscopy (Section 3.4.13.2), protein assay (Section 3.4.13.3) and biosorption efficiency (Section 3.4.13.4). To compare the change of PCO treated biosorbents with untreated biosorbents, the freeze-dried biosorbents mentioned in Section 3.1.2 were also utilized for analysis.

3.4.13.1 Chitin assay

The chitin contents of PCO treated and untreated chitin A and chitin B were determined gravimetrically after removing the protein and the minerals based on the chitin assay mentioned by Ferrer *et al.* (1996). The weighed oven-dried biosorbents (W_b) were first deproteinized by 2 N NaOH in a ratio of 1:20 (w/v) for a few minutes to form alkaline insoluble materials (AIM). Then the AIM fraction was collected by filtration with GF/C filter paper and neutralized by ultrapure water. After that it was demineralized using 2 N HCl (Univar, Seven-Hills, Australia) in a ratio of 1:10 (w/v) agitated by an orbital shaker at 200 rpm for 1 h in order to extract out the chitin fraction. This chitin residue was filtered using a pre-weighed oven-dried Whatman 42 filter paper (Kent, UK). And the filter residue was washed with ultrapure water and dried in an oven at 105°C for 24 h. The percentage of chitin content of biosorbents (w/w) was then calculated using the following equation.

$$\text{Chitin content (\%)} = (W_{c+f} - W_f) / W_b \times 100 \qquad (3.6)$$

where W_{c+f} is the dry weight of filter paper loaded with chitin residue (g), W_f is the dry weight of filter paper (g) and W_b is the dry weight of biosorbent (g).

3.4.13.2 Diffuse reflectance Fourier transform infra-red spectroscopy

The structures of PCO treated and untreated biosorbents were determined by diffuse reflectance Fourier transform infra-red (DRFT-IR) spectroscopy (Pecchi *et al.*, 2001; Yang *et al.*, 2001). DRFT-IR spectra of biosorbents in the wave number region between 4,000 and 400 cm^{-1} were recorded using a Nicolet Magna 560 FT-IR spectrometer (Madison, USA) equipped with OMNIC FT-IR software (Nicolet).

The PCO treated biosorbents were firstly lyophilized by a freeze-dryer (Labconco, Kansas City, USA) at 0°C at a reduced pressure for 5 days as described in Section 3.1.2. Then the untreated and PCO treated biosorbents were ground into a fine powder with mortar and pestle and then mixed with anhydrous potassium bromide (Merck, Darmstadt, Germany) in the ratio of 1:100 (w/w) (Sandula *et al.*, 1999; Tsui, 2000). The mixtures were poured into the micro-sampler and pressed into flat and smooth surfaces. The spectra were all recorded at 4 cm^{-1} resolutions and averaged over 32 scans with dynamic background subtraction. In addition, the

Table 3.3 Analytical conditions for the gas chromatography/mass spectrum analysis (modified from Chiu *et al.*, 1998, Fong, 2001).

Injection	Carrier gas	Helium (He)
	Total flow rate	24.7 mL/min
	Port temperature	250°C
Column	Pressure	5.5
	Flow	0.7 mL/s (constant)
	Average velocity	31 cm/s
Oven	Oven programme	80°C for 1 min, increase at 10°C/min, keep at 200°C for 15 min

roughly structures of PCP adsorbed chitin A with PCO reaction irradiated for 0, 120, 240, 360 and 480 min as described in Section 3.4.7 were also analyzed with DRFT-IR to examine the effect of prolonging PCO reaction.

3.4.13.3 Protein assay

The protein contents of the PCO treated and untreated biosorbents were determined so as to analyze the composition of the biosorbents and to investigate how much protein in the biosorbents was degraded by PCO. This was achieved by mixing 0.1 g of individual biosorbent with 2 mL of ultrapure water and the pH of biosorbent solution was adjusted to 12 using 2 N NaOH in order to dissolve the protein present. The biosorbent solution was agitated by an orbital shaker at 200 rpm for an extracton time of 2 hr (Ferrer *et al.*, 1996). After that, the biosorbent was separated by a Sanyo MicroCentaur (Loughborough, England) at 13,000 rpm for 3 min and the supernatant was pipetted out and analyzed by the modified Lowry method (Sigma Diagnostics® procedure No. 690) with protein assay kid (Sigma Chemicals, St. Louis, USA). The absorbance of supernatant was measured at a wavelength of 725 nm by spectrophotometer (Milton Roy Spectronic 601, New York, USA). The solubilized protein was turned into blue colour once mixed with the supernatant with a diluted biuret reagent (Sigma Chemicals) and Folin and Ciocalteu's Phenol reagent (Sigma Chemicals) following the protocol provided in the kit. Protein content was then determined from a standard curve.

3.4.13.4 Biosorption efficiency

The biosorption efficiency of PCO treated and untreated biosorbents were investigated under the same optimal conditions mentioned in Section 3.3. The PCO treated biosorbents were first freeze dried as mentioned in Section 3.1.2. Then 0.4 g dry weight of PCO treated and untreated biosorbents were immersed into 50 mL of 10 mg/L PCP solution for 60 min respectively. The RE was then found as described in Section 3.3 and compared with one another.

3.4.14 Multiple biosorption and PCO cycles of PCP

Batch type multiple biosorption and PCO cycles of PCP by chitin A were determined in order to establish whether the biosorbents could be regenerated. Based

on the results in Section 3.4.7, the degradation of PCP was almost completed at 240 min irradiation time. Thus, the biosorbents from two and four of 10 mg/L PCP biosorption batches (0.8 g and 1.6 g), as mentioned in Section 3.3.3.3, were brought to 240 min PCO reaction respectively. Then these PCO treated biosorbents were collected by filtration by GF/C filter paper and immersed into another 50 mL of 10 mg/L of PCP solution as aforementioned in Section 3.3. After that they were put for PCO reaction again and these cycles were repeated four times.

3.4.15 Evaluation for the toxicity change of PCP adsorbed biosorbents during PCO

Vibrio fisheri was employed as the testing organism to evaluate the toxicity of PCP adsorbed biosorbents with Solid-phase Microtox® test system consisted of a Microbics M500 Microtox® analyzer (Microbic Corporation, Carlsbad, USA). Median effective concentration (EC50) of the samples was automatically calculated by a Microtox® Data Collection and Reduction System (Microbic Corporation, Carlsbad, USA) (Bolduc & Anderson, 1997; Cauntú *et al.*, 2000).

The test was performed following the protocol of Microtox® Manual for the solid-phase test in which 0.3 g of wet PCP adsorbed biosorbents obtained from PCO batches in Section 3.4.9 (0.4 g of chitin A originally immersed in 10 mg/L of PCP biosorption batch) was applied for the toxicity test. To estimate the detoxifying ability of PCO, the toxicities of chitin A and PCO treated chitin A were also evaluated for comparison. EC50-5min and EC50-15min were studied to confirm the nature of sample.

4. Results

4.1 Batch biosorption experiment

4.1.1 Selection of optimal conditions for batch PCP adsorption

The PCP removal efficiencies and removal capacities of chitin A, chitin B and chitosan in different batch experiment were determined. The results showing the effect of initial pH, biosorbent concentration, temperature and agitation rate are presented below.

4.1.1.1 Effect of initial pH and biosorbent concentration

Figures 4.1, 4.2 and 4.3 show the effect of initial pH of PCP solution at different contact time of 0.2, 0.4 and 0.8 g of chitin A, chitin B and chitosan respectively on removal efficiency. From the preliminary results, it showed that the deviations on PCP REs obtained from biosorption with 0.025, 0.05 and 0.1 g of biosorbents were too large, and PCP REs performed by this range of biosorbent concentrations were too limited (data not shown); only the effects of 0.2, 0.4 and 0.8 g of biosorbents are shown.

The trends of three biosorbents were similar, in which RE increased with the biosorbent concentrations regardless of initial pH. Except the differences among the three biosorbent concentrations at initial pH 2.5 of chitin B, it showed no obvious effect of biosorbent concentration. The RE increased sharply at first 15 min, and gradually increased to 45 min. It plateaued at 60 min, and after that, there was no significant increase on the RE (data not shown here). In addition, the initial pH effect of 0.4 g of biosorbent concentration, except chitin B at initial pH 2.5, was more obvious (RE ranged from 65 to 80% for chitin A; 25 to 65% for chitin B and 35 to 50% for chitosan) than that of 0.2 and 0.8 g (RE ranged from 50 to 60% and 81 to 87% for 0.2 and 0.4 g of chitin A respectively; 10 to 59% and 50 to 67% for 0.2 and 0.4 g of chitin B; 23 to 27% and 59 to 68% for 0.2 and 0.4 g of chitosan). Therefore, 0.4 g of biosorbent concentration was chosen in the subsequent experiments.

To examine the effect more easily and clearly, the 2-D graphs (Figures 4.4 and 4.5) were used, in which the effects solely at 60 min of 0.4 g of biosorbents were presented on removal efficiency and removal capacity. From Figure 4.4, it was obvious that REs of chitin A were highest, followed by chitosan and chitin B except at initial pH 2.5, in which RE of chitin B was higher than chitosan. And the final pH

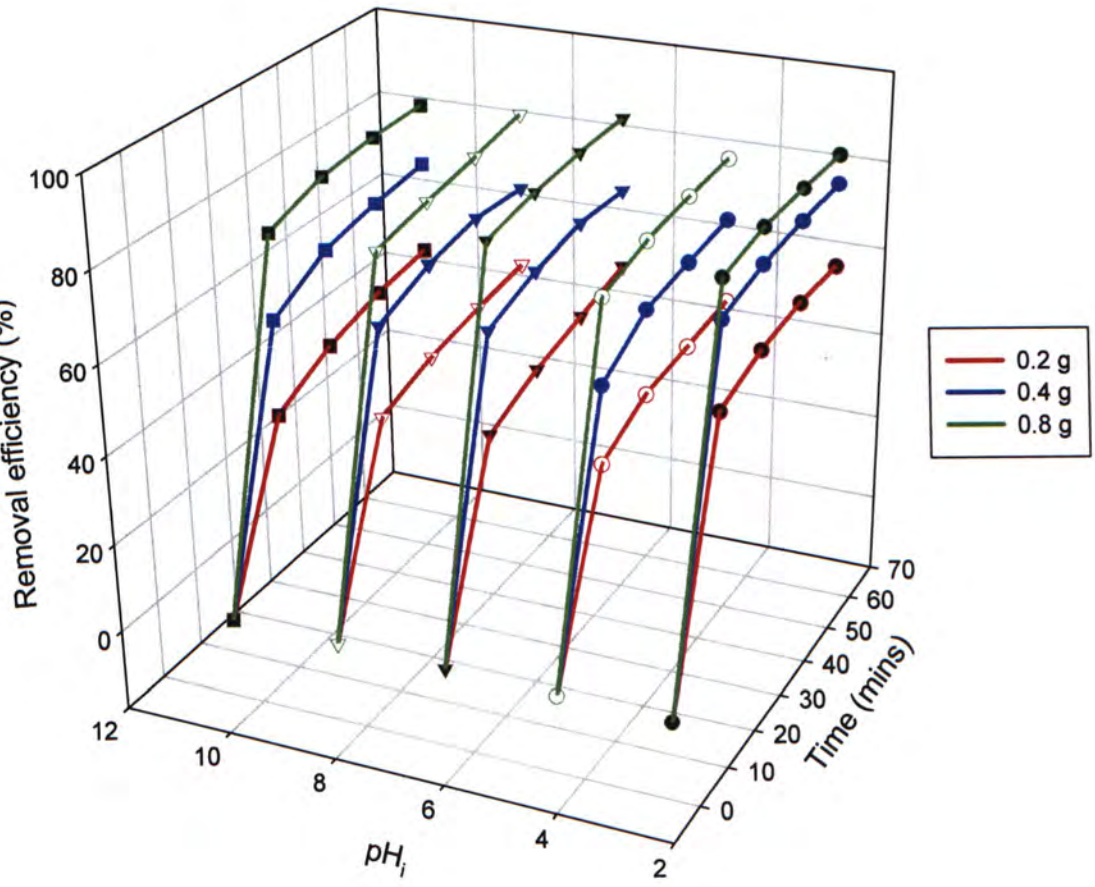


Figure 4.1 The PCP removal efficiency of 0.2, 0.4 and 0.8 g of chitin A at different initial pH of PCP solution. The experimental conditions: concentration of PCP solution = 10 mg/L, agitation rate = 200 rpm, temperature = 25°C, solution volume = 50 mL. Data are the means of triplicate.

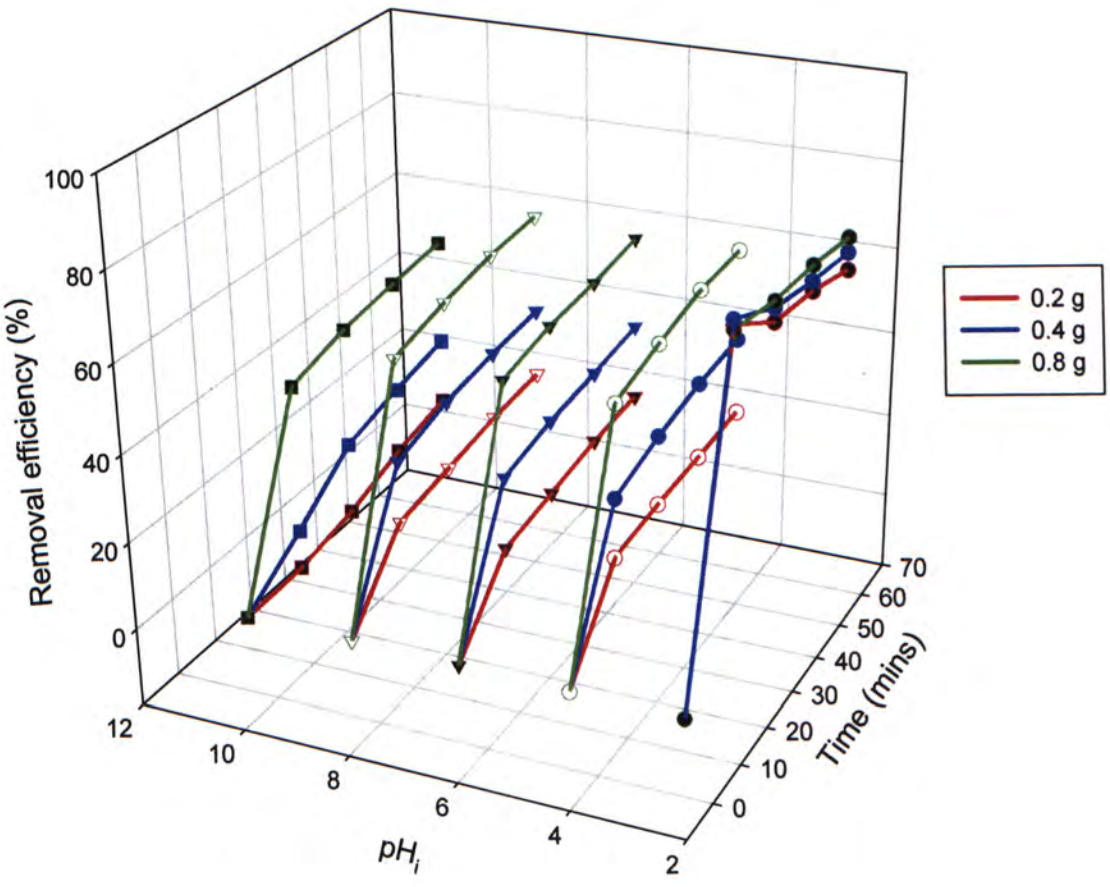


Figure 4.2 The PCP removal efficiency of 0.2, 0.4 and 0.8 g of chitin B at different initial pH of PCP solution. The experimental conditions: concentration of PCP solution = 10 mg/L, agitation rate = 200 rpm, temperature = 25°C, solution volume = 50 mL. Data are the means of triplicate.

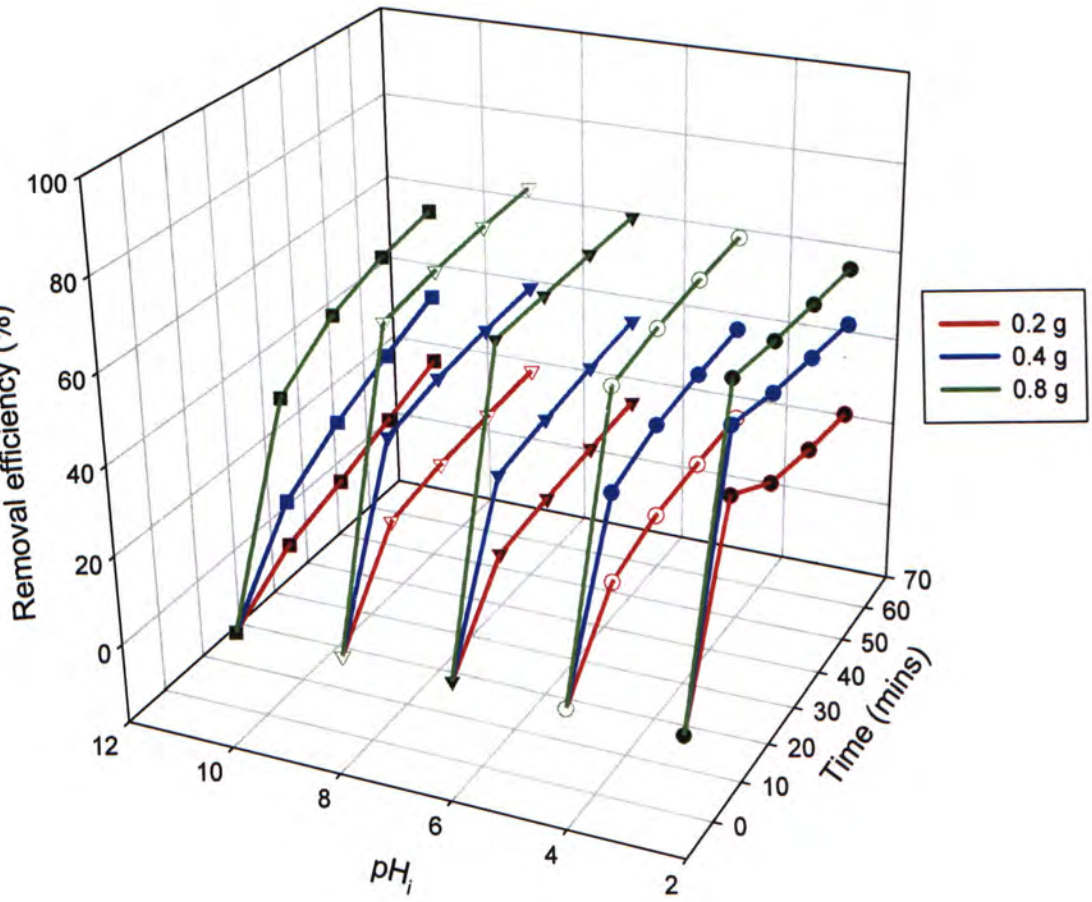


Figure 4.3 The PCP removal efficiency of 0.2, 0.4 and 0.8 g of chitosan at different initial pH of PCP solution. The experimental conditions: concentration of PCP solution = 10 mg/L, agitation rate = 200 rpm, temperature = 25°C, solution volume = 50 mL. Data are the means of triplicate.

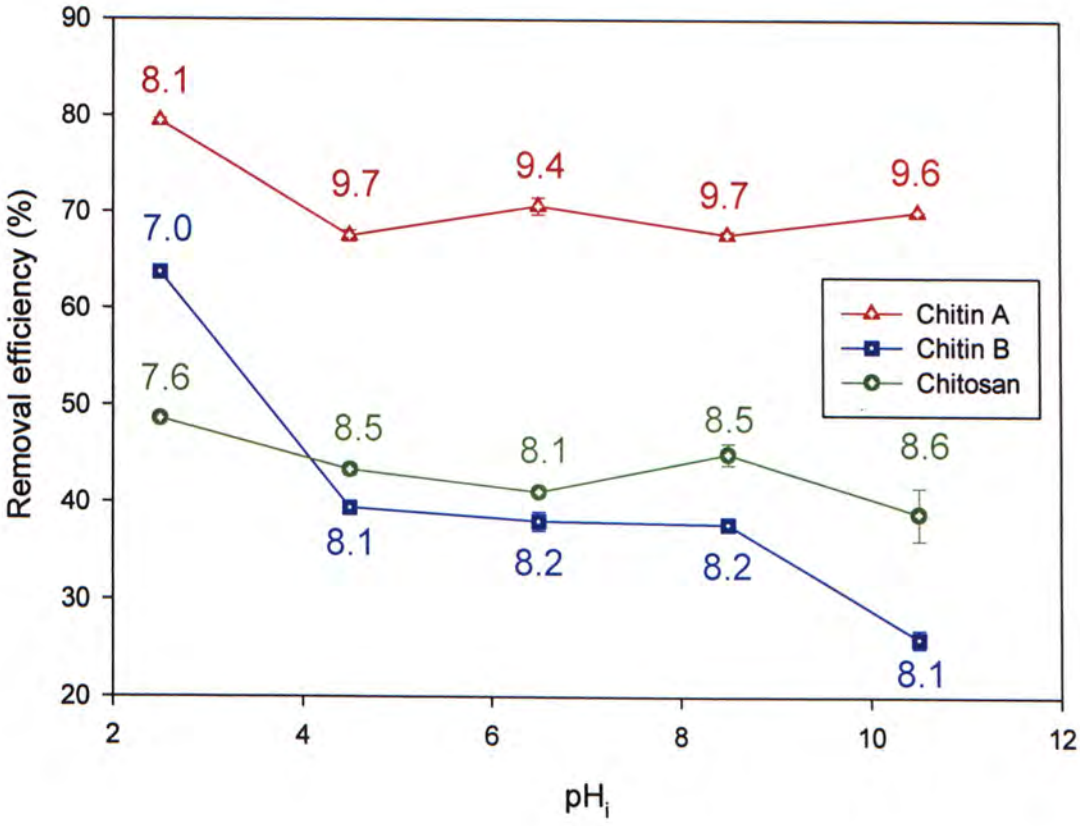


Figure 4.4 The PCP removal efficiencies of 0.4 g of chitin A, chitin B and chitosan at different initial pH of PCP solution at 60 min. The experimental conditions: concentration of PCP solution = 10 mg/L, agitation rate = 200 rpm, temperature = 25°C, solution volume = 50 mL. Data represent the means and error bars represent the standard deviations of triplicates. The number represented the final pH of the sample at 60 min.

of the samples reached at some extend constant after the addition of biosorbents, even after 24 h (data not shown).

From Figure 4.5, it was shown that PCP RCs of biosorbents decreased with the increase of initial pH. All three biosorbents showed the highest RC at initial pH 2.5, and no big differences at initial pH 4.5 to 8.5. For chitin B and chitosan, RCs were the lowest at extreme alkaline condition, but no obvious effect on chitin A.

4.1.1.2 Effect of Tris buffer and biosorbent concentrations

Figures 4.6, 4.7 and 4.8 showed REs and RCs with using Tris buffer at pH 6.5 of chitin A, chitin B and chitosan respectively. It was found that keeping the solution pH at constant 6.5 could yield higher REs and RCs. The RE of 0.8 g of chitin A reached approximately 100% at first 15 min and 90% for 0.4 g. For the RC, Tris buffer could enhance 22%, compared with that of 0.4 g of chitin A in solution with initial pH 6.5. On the contrary, REs and RCs increased 36% and 34% for chitin B and chitosan respectively when Tris buffer was used. In addition, from the figures, it also expressed that higher biosorbent concentrations resulted in increasing REs, but decreasing RCs.

4.1.1.3 Effect of temperature

The effect of temperature of samples on PCP adsorption was presented in Figure 4.9. It showed that REs and RCs of three biosorbents at 22°C and 25°C were statistically identical; whereas they decreased with the increase of temperature. Thus subsequent experiments were performed at room temperature.

4.1.1.4 Effect of agitation rate

PCP REs and RCs of chitin A, chitin B and chitosan were determined under the same conditions apart from varying the agitation rate, and the results were demonstrated in Figure 4.10. The results showed that the agitation rate did not significantly influence the performance of biosorption. And thus, 200 rpm was chosen as selected condition.

By Integrating the above results, the common optimal conditions (initial pH of PCP solution, biosorbent concentration, contact time, temperature and agitation rate) for chitin A, chitin B and chitosan were obtained and expressed in Table 4.1.

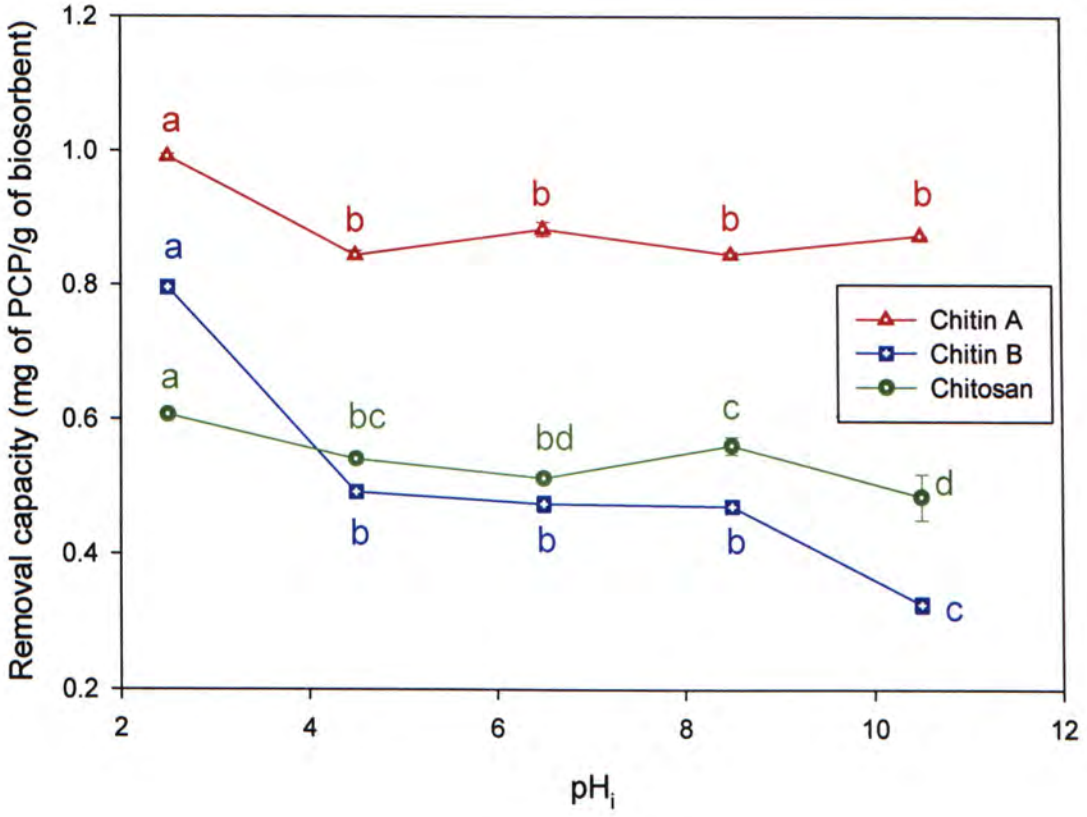


Figure 4.5 The PCP removal capacities of 0.4 g of chitin A, chitin B and chitosan at different initial pH of PCP solution at 60 min. The experimental conditions: concentration of PCP solution = 10 mg/L, agitation rate = 200 rpm, temperature = 25°C, solution volume = 50 mL. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

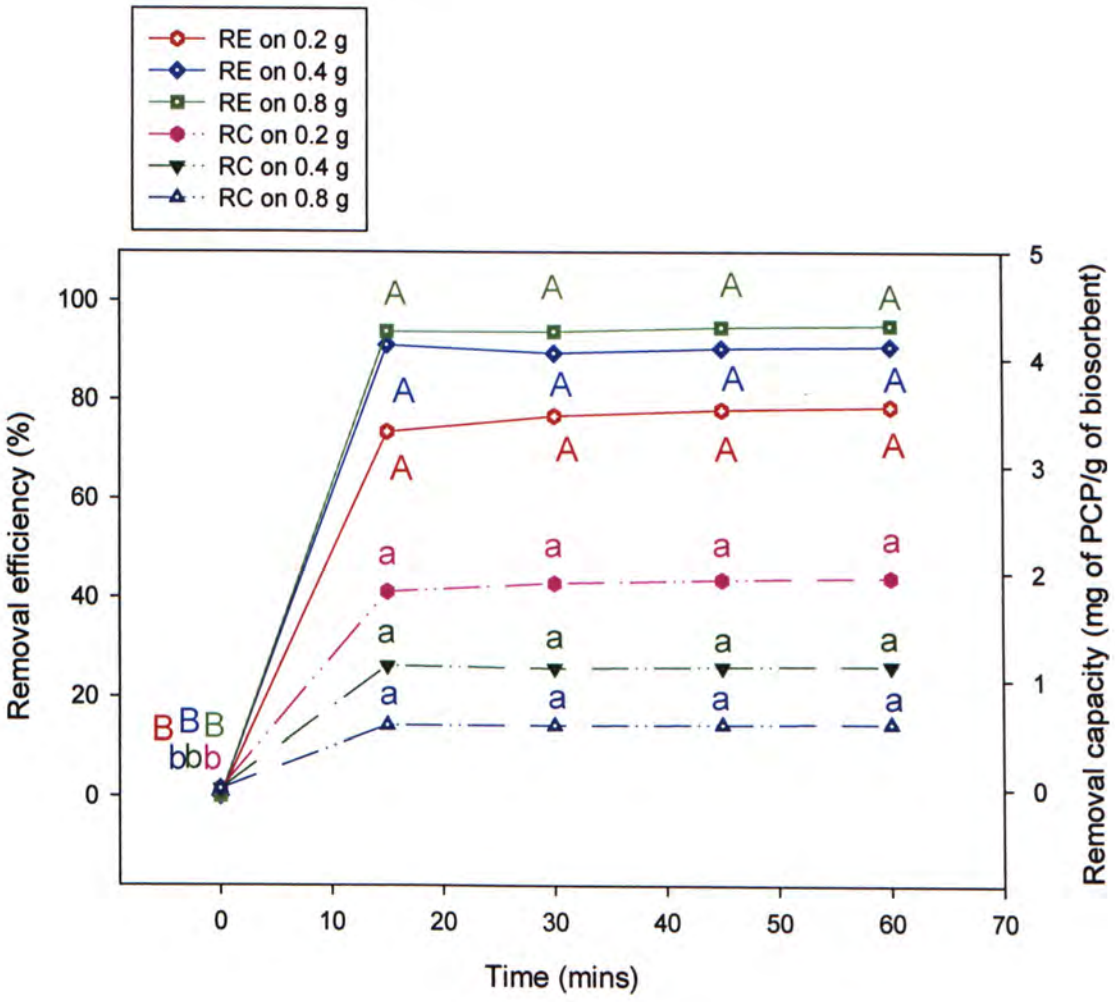


Figure 4.6 The PCP removal efficiencies (REs) and removal capacities (RCs) of 0.2, 0.4 and 0.8 g of chitin A at Tris buffering pH 6.5 of PCP solution changing with time. The experimental conditions: concentration of PCP solution = 10 mg/L, agitation rate = 200 rpm, temperature = 25°C, solution volume = 50 mL. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

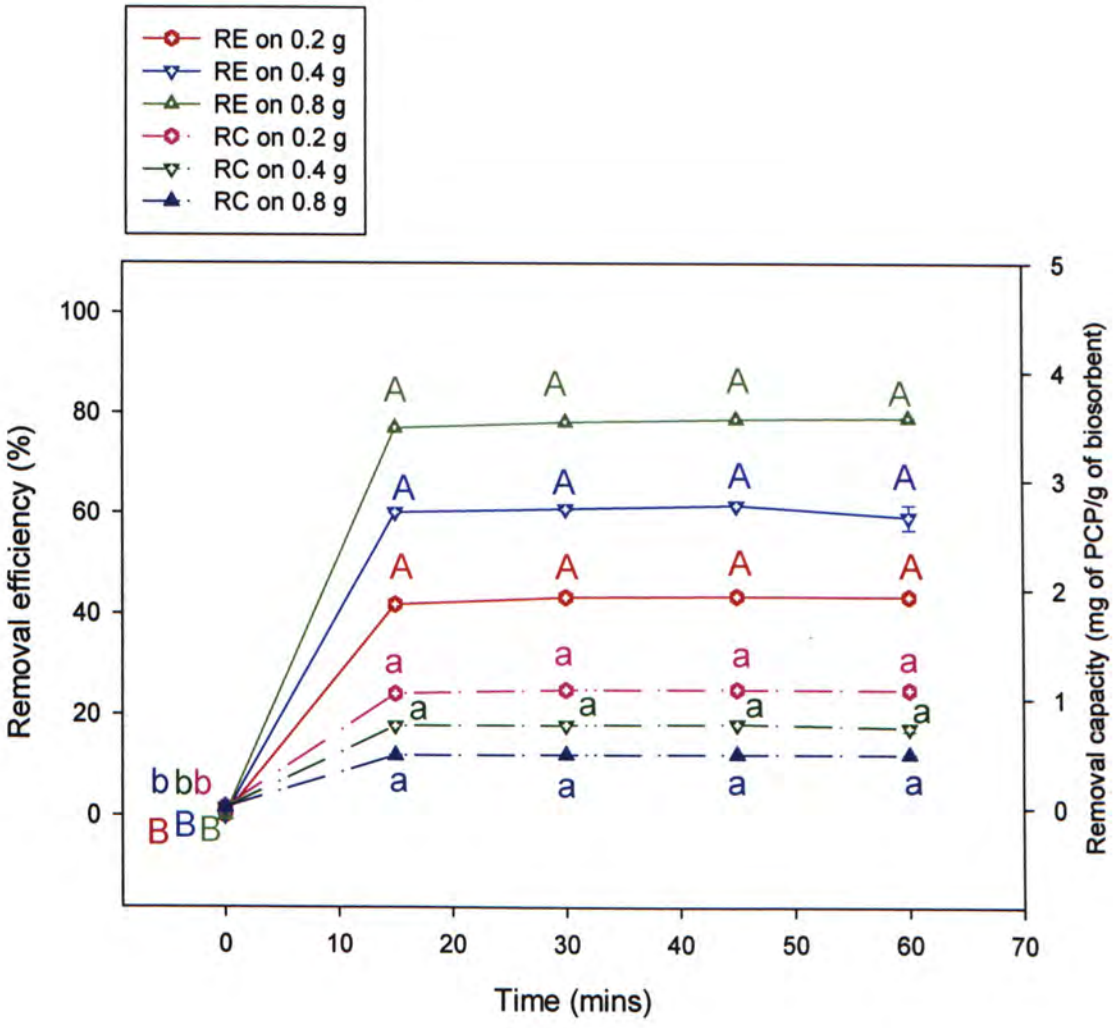


Figure 4.7 The PCP removal efficiencies (REs) and removal capacities (RCs) of 0.2, 0.4 and 0.8 g of chitin B at Tris buffering pH 6.5 of PCP solution changing with time. The experimental conditions: concentration of PCP solution = 10 mg/L, agitation rate = 200 rpm, temperature = 25°C, solution volume = 50 mL. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

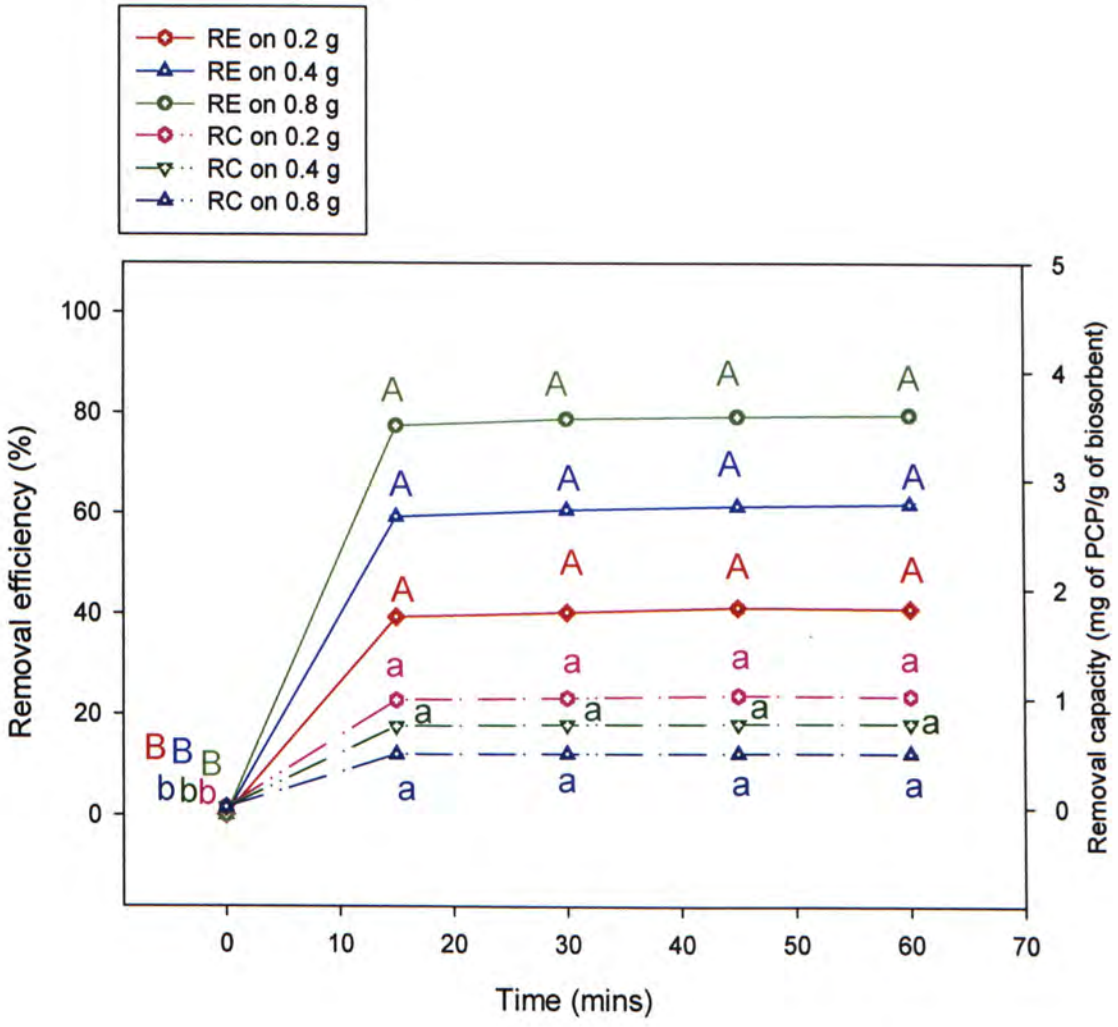
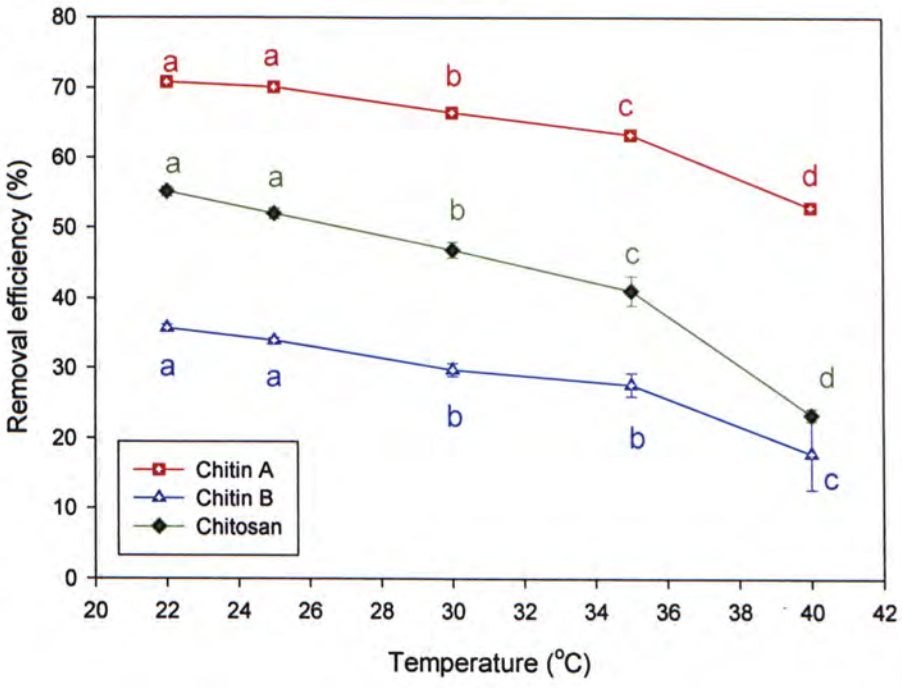


Figure 4.8 The PCP removal efficiencies (REs) and removal capacities (RCs) of 0.2, 0.4 and 0.8 g of chitosan at Tris buffering pH 6.5 of PCP solution changing with time. The experimental conditions: concentration of PCP solution = 10 mg/L, agitation rate = 200 rpm, temperature = 25°C, solution volume = 50 mL. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

(a)



(b)

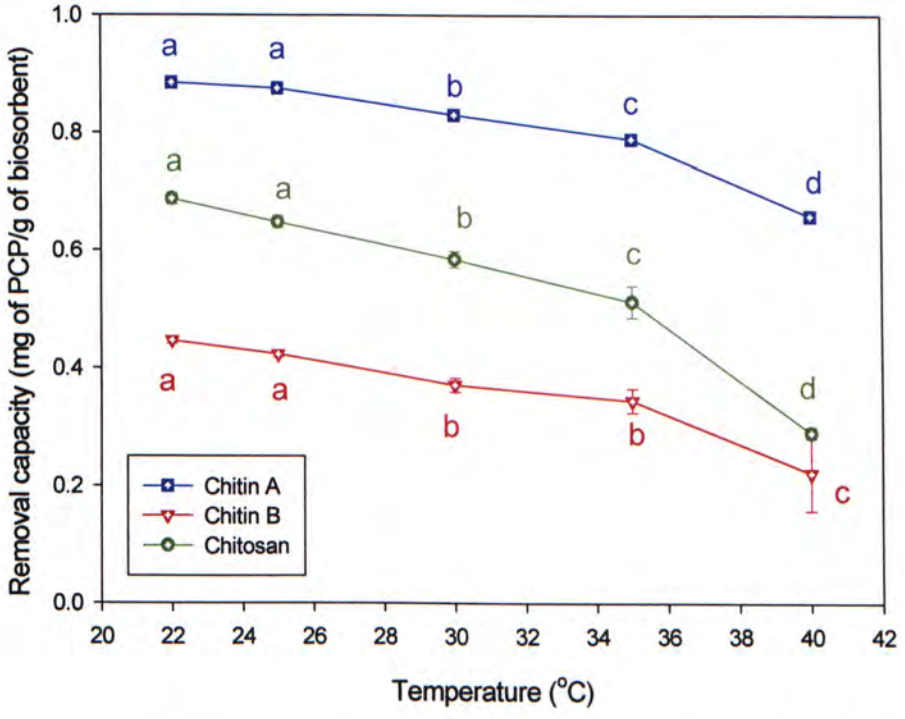


Figure 4.9 The (a) PCP removal efficiencies (REs); and (b) PCP removal capacities (RCs) of biosorption changing with temperature of chitin A, chitin B and chitosan. The experimental conditions: concentration of PCP solution = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL, agitation rate = 200 rpm, contact time = 60 min. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

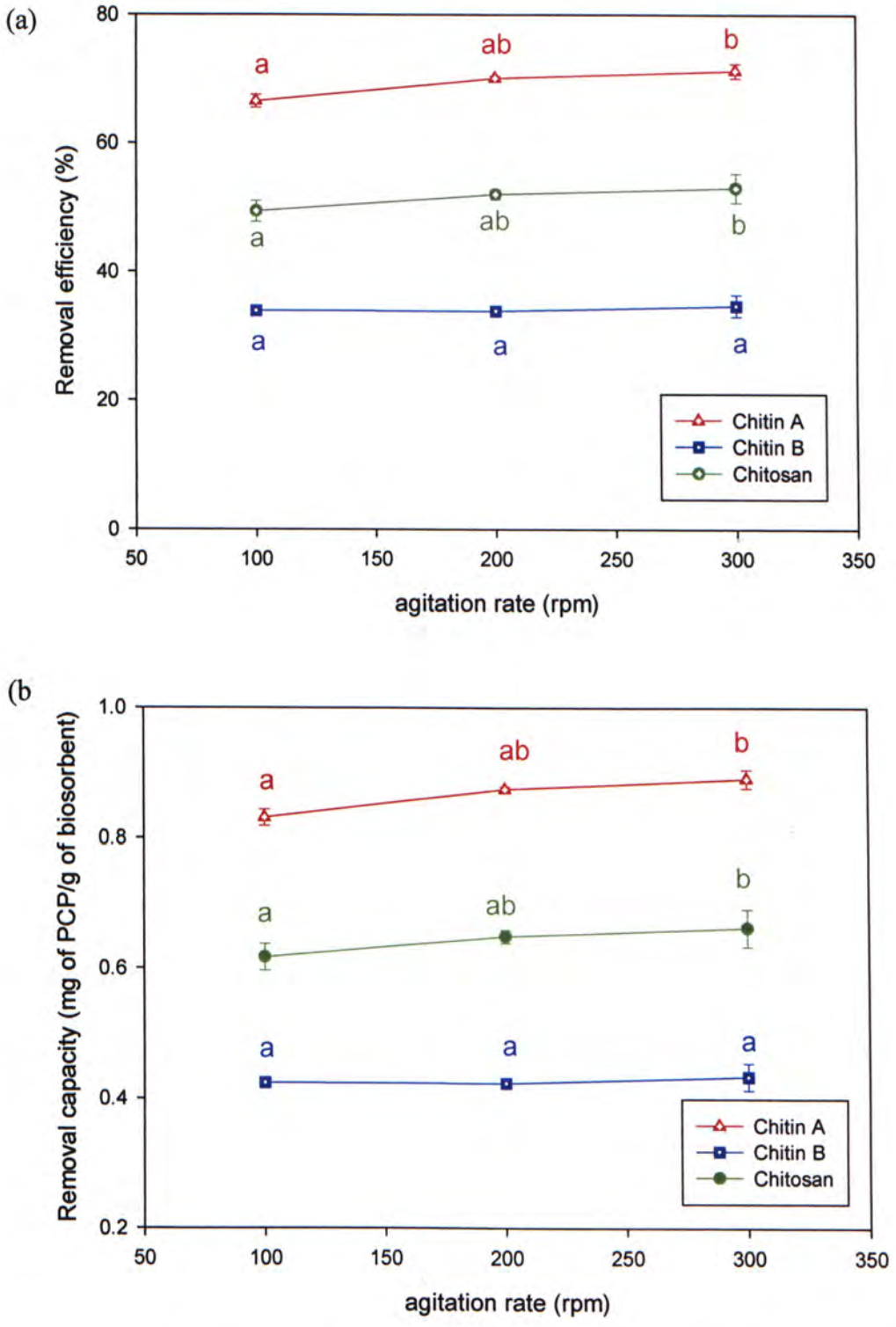


Figure 4.10 The (a) PCP removal efficiencies (REs); and (b) PCP removal capacities (RCs) of biosorption changing with agitation rate of chitin A, chitin B and chitosan. The experimental conditions: concentration of PCP solution = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL, temperature 23±2°C, contact time = 60 min. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

Table 4.1 The selected conditions for biosorption with chitin A, chitin B and chitosan.

Parameter	**Selected condition**
Initial pH of PCP solution	6.5
Biosorbent concentration	8 mg/mL (0.4 g/50 mL)
Contact time	60 min
Temperature	23 ± 2°C
Agitation rate	200 rpm

4.1.2 Effect of initial PCP concentration and biosorbent concentration

The effect of initial PCP concentrations and biosorbent concentrations on chitin A, chitin B and chitosan were shown in Figures 4.11, 4.12 and 4.13. It appeared that the PCP uptake capacity was obviously influenced by the initial concentrations of PCP. Among the three graphs showing RC (Figures 4.11b, 4.12b and 4.13b), the capacity increased sharply with the increase of initial PCP concentrations, especially in the case of chitin A (with 11 mg of PCP/g of biosorbents increased from 5 mg/L of PCP to 300 mg/L). For RCs of chitin B and chitosan, it was shown that the biosorbent concentration was independent to the PCP concentration, and it reached plateau at high PCP concentrations (200 and 300 mg/L) (Figures 4.12 and 4.13). On the other hand, RE increased with biosorbent concentration proportionally, but decreased with PCP concentration. However, while considering the case in chitin A, RCs increased with less biosorbents used regardless of the concentration of PCP. In addition, a plateau could not be reached within the concentrations of PCP studied (5–300 mg/L) (Figure 4.11).

4.1.2.1 Adsorption isotherm

Two commonly used monolayer adsorption models, Langmuir and Freundlich adsorption isotherms, are employed to describe the PCP adsorption phenomenon of biosorbents. The results from Section 4.1.2 were transformed into the equations of two monolayer models stated in Section 1.2.3, and hence the graphs were obtained and described in Figures 4.14 and 4.15. The Langmuir and Freundlich constants obtained from linearized plots are given in Table 4.2.

Based on the correlation coefficients, it is obvious that the adsorption equilibrium data for all three biosorbents fitted Langmuir isotherm (Figure 4.15) better than Freundlich isotherm (Figure 4.16), while only chitin B showed good-fitted to Freundlich. By comparison of the adsorption constants, chitin A had the highest adsorption capacity (q_{max} and k) and affinity (b) for PCP. However, for the intensity constant (n), chitosan was the highest. On the other hand, when considering the relationship between biosorbent concentrations and adsorption constants, it seemed that the factor of biosorbent concentrations is independent on the isotherm model. No obvious correlation could be concluded.

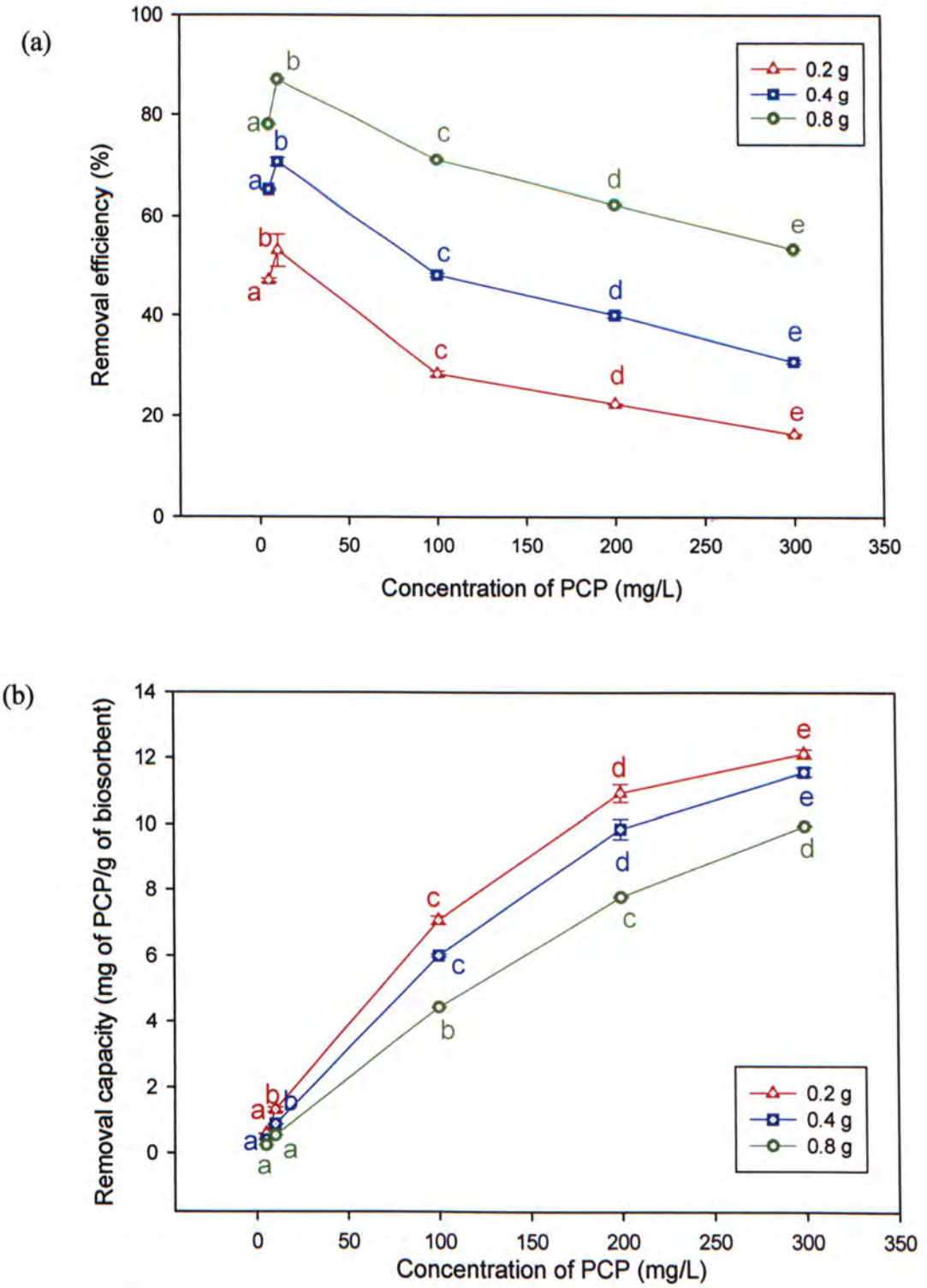
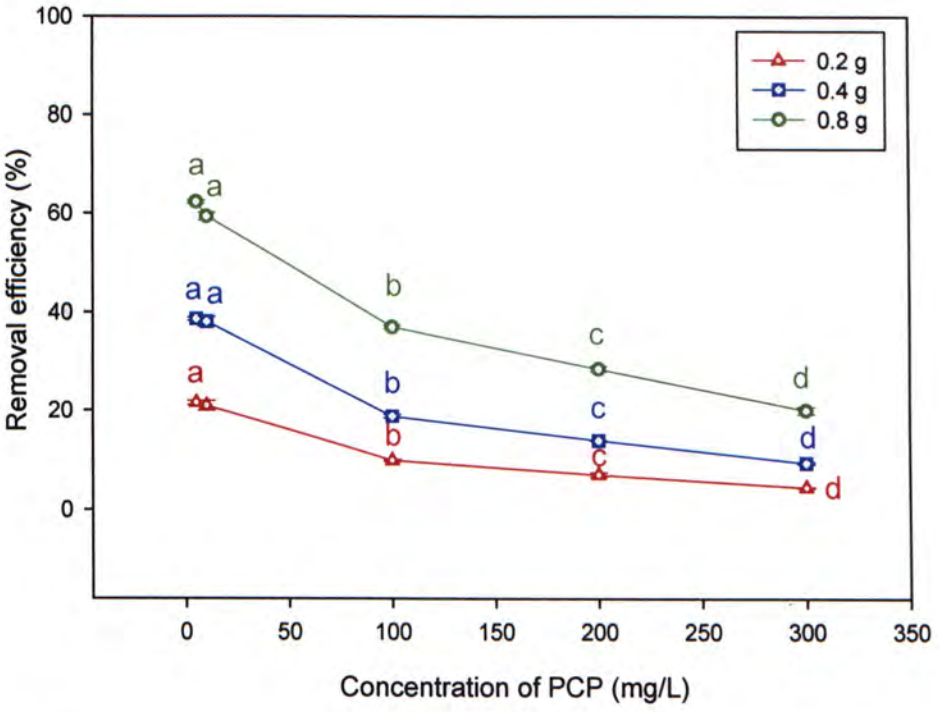


Figure 4.11 The (a) PCP removal efficiencies (REs); and (b) PCP removal capacities (RCs) of biosorption changing with PCP concentration and biosorbent concentration of chitin A. The experimental conditions: initial pH = 6.5, reaction volume = 50 mL, temperature 23±2°C, contact time = 60 min, agitation rate = 200 rpm. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

(a)



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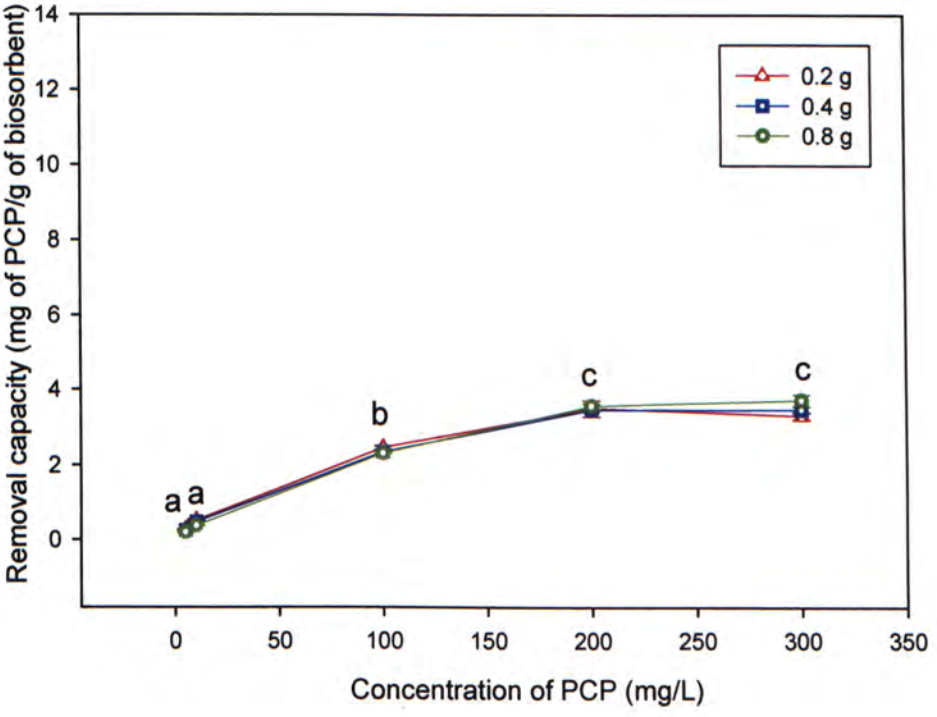
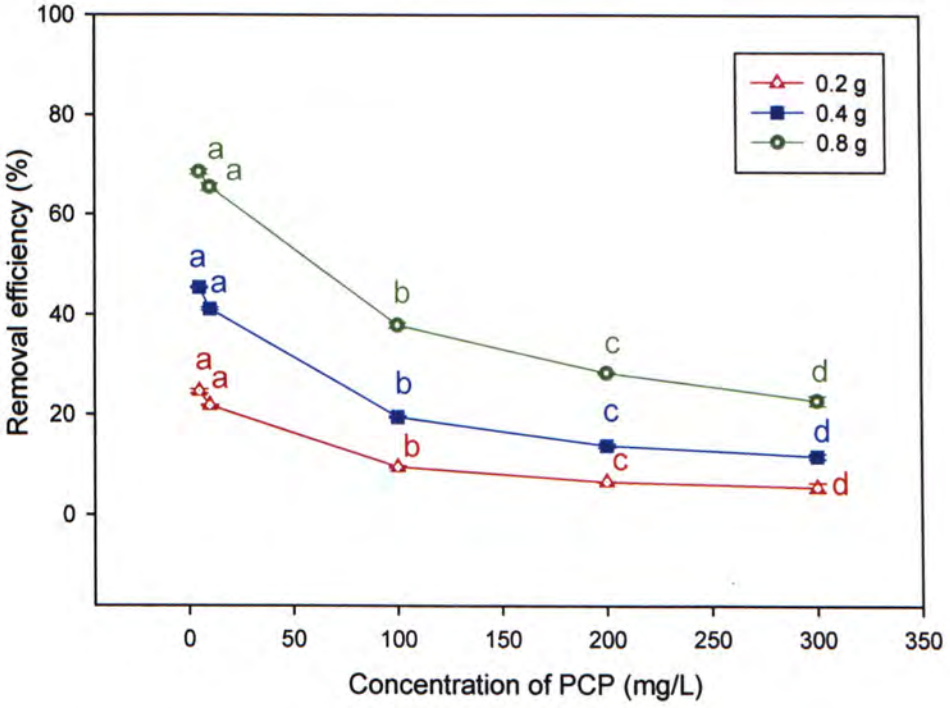


Figure 4.12 The (a) PCP removal efficiencies (REs); and (b) PCP removal capacities (RCs) of biosorption changing with PCP concentration and biosorbent concentration of chitin B. The experimental conditions: initial pH = 6.5, reaction volume = 50 mL, temperature 23±2°C, contact time = 60 min, agitation rate = 200 rpm. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$). For Figure (b), all statistical results among the biosorbent concentrations were the same.

(a)



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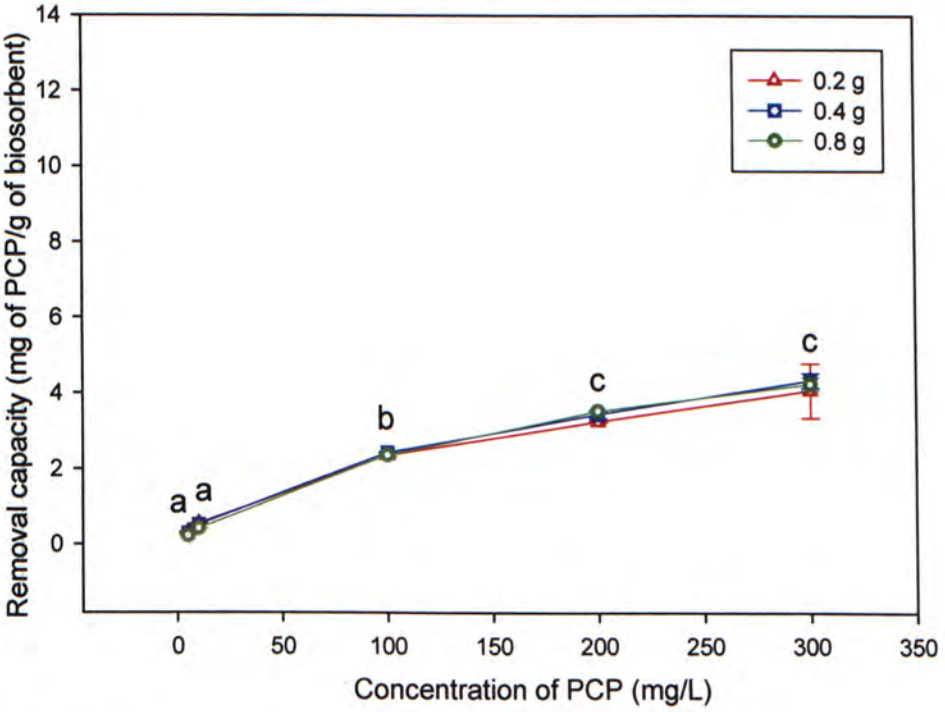

Figure 4.13 The (a) PCP removal efficiencies (REs); and (b) PCP removal capacities (RCs) of biosorption changing with PCP concentration and biosorbent concentration of chitosan. The experimental conditions: initial pH = 6.5, reaction volume = 50 mL, temperature 23±2°C, contact time = 60 min, agitation rate = 200 rpm. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$). For Figure (b), all statistical results among the biosorbent concentrations were the same.

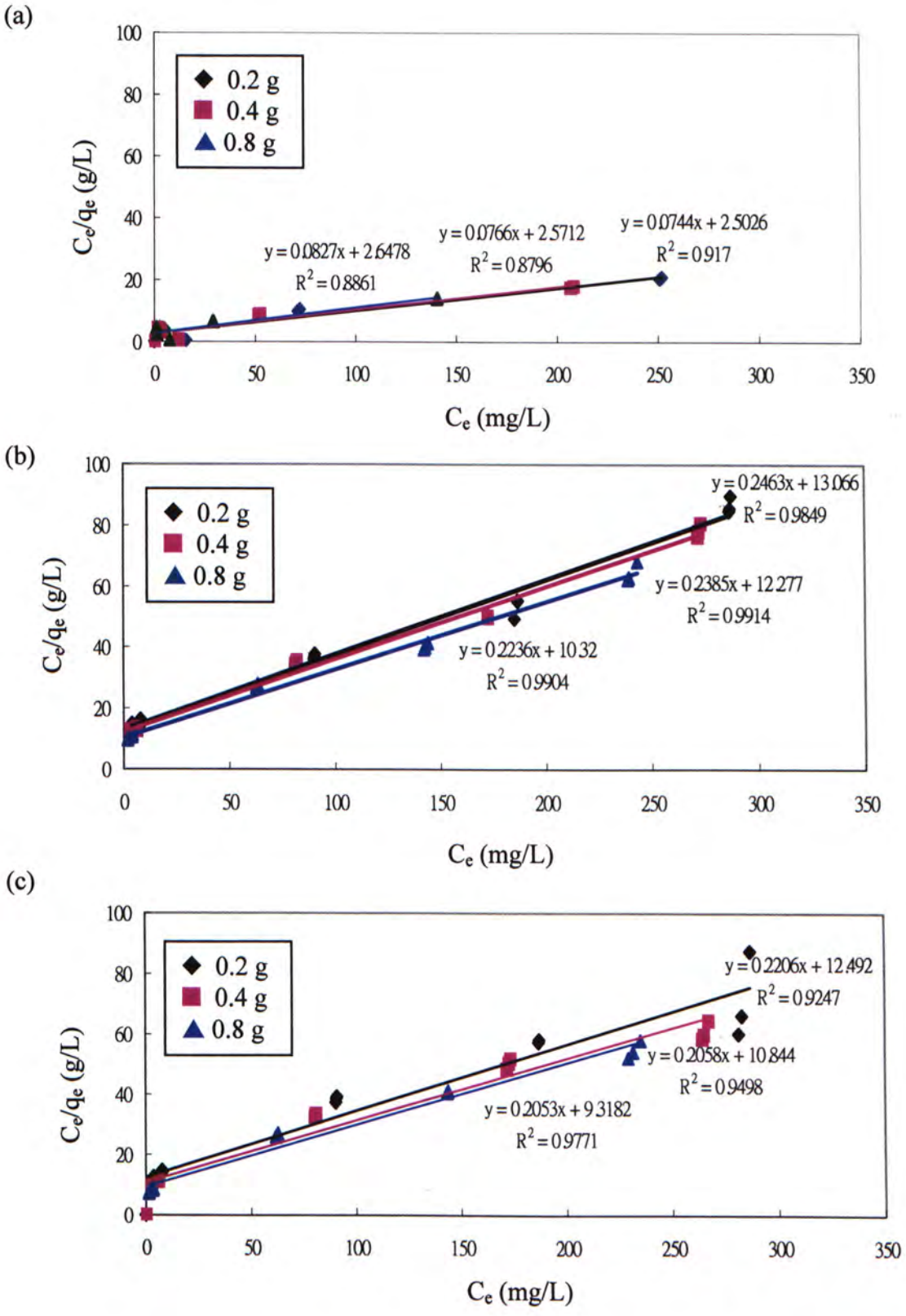

Figure 4.14 Langmuir isotherm of PCP adsorption by 0.2 (◆), 0.4 (■) and 0.8 g (▲) of (a) chitin A, (b) chitin B and (c) chitosan.

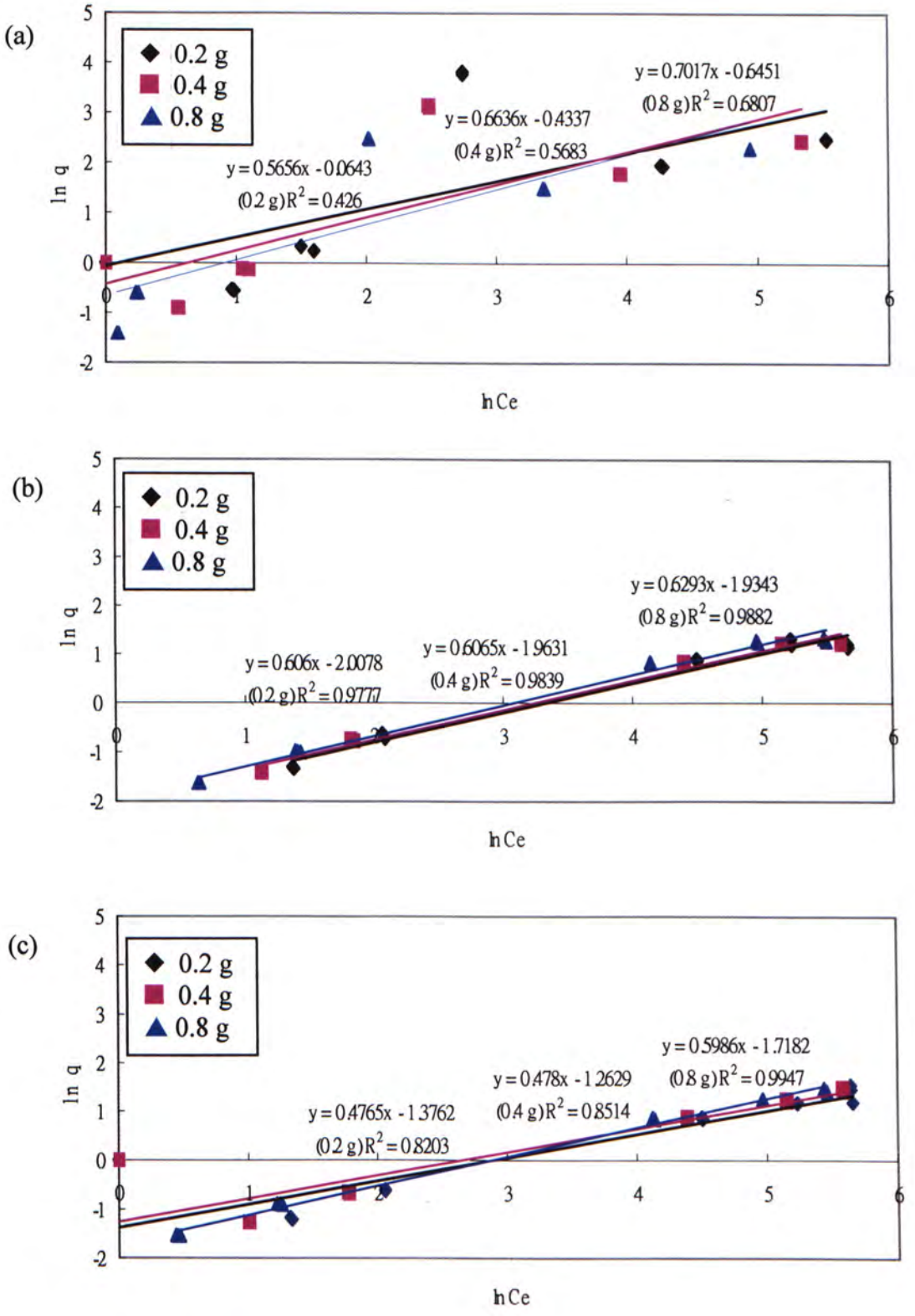

Figure 4.15 Freundlich isotherm of PCP adsorption by 0.2 (◆), 0.4 (■) and 0.8 g (▲) of (a) chitin A, (b) chitin B and (c) chitosan.

Table 4.2 Langmuir and Freundlich isotherm constants and correlation coefficients for PCP adsorption by 0.2, 0.4 and 0.8 g of chitin A, chitin B and chitosan.

	Amount of biosorbent (g/50 mL)	Langmuir Isotherm			Freundlich Isotherm		
		q_{max} (mg/g)	b (L/mg)	r^2	k	n	r^2
Chitin A	0.2	13.441	0.030	0.917	0.938	1.768	0.426
	0.4	13.055	0.030	0.880	0.648	1.507	0.568
	0.8	12.092	0.031	0.887	0.524	1.425	0.681
Chitin B	0.2	4.060	0.019	0.985	0.134	1.650	0.978
	0.4	4.193	0.019	0.991	0.140	1.649	0.984
	0.8	4.472	0.022	0.990	0.145	1.589	0.988
Chitosan	0.2	4.533	0.017	0.925	0.252	2.099	0.820
	0.4	4.859	0.019	0.950	0.283	2.092	0.851
	0.8	4.971	0.022	0.977	0.179	1.671	0.995

4.2 Photocatalytic oxidation

4.2.1 Selection of extraction solvent

Based on the preliminary results, the water-soluble extraction solutions (NaOH and MeOH) were chosen as potential candidates for extracting PCP from the biosorbent, chitin A. In order to obtain higher extraction efficiency, the most suitable concentration of extraction solutions (0.05, 0.5, 1, 5, 10 and 25 mM of NaOH and 25, 50, 75 and 100% of MeOH) was investigated and the results were shown in Figure 4.16. From the figure, it was shown that methanol (MeOH) showed higher extraction efficiency than that of NaOH except 25% of MeOH. It showed that the extraction efficiency was lower at extremely low and high NaOH concentrations (50μM and 25mM). For using MeOH, the extraction efficiency was increased with the concentration, and reached the highest efficiency with 94% for 75% MeOH. However, the extraction using pure MeOH was not preferred. Therefore, 75% of MeOH was selected for extraction of PCP from chitin A in subsequent experiments.

4.2.2 Determination of hydrogen peroxide concentration

The consumption of hydrogen peroxide (H_2O_2) in the reaction was monitored in order to determine the time for recruitment to ensure the unlimited supply of H_2O_2 for the further experiments. It could be observed from Figure 4.17 that H_2O_2 was quickly consumed in first hour, and still remained at low concentration after 4 h (21 μM). After 6 h, all $H_2O_2$was used. Therefore, it was determined that H_2O_2 (6.7 mM) should be added in the forth hour of the reaction.

4.2.3 Effect of biosorbent concentration in PCO

The biosorbents, with concentration 0.4, 0.8 and 1.6 g, obtained from batch experiment under the selected conditions were collected and pooled for PCO reactions respectively under the same selected conditions. The distribution of PCP in the reaction mixture solution for PCO and ultrapure water in control system was demonstrated in Figures 4.18, 4.19 and 4.20. By concerning the distribution of PCP in control systems (Figures 4.18b, 4.19b and 4.20b), it was observed that PCP firstly diffused from biosorbents to the aqueous medium and remained at constant at very low level, which was called apparent-equilibrium. This phenomenon was prominent when considering 0.4 g of biosorbent in control system (Figure 4.18b). Provided that

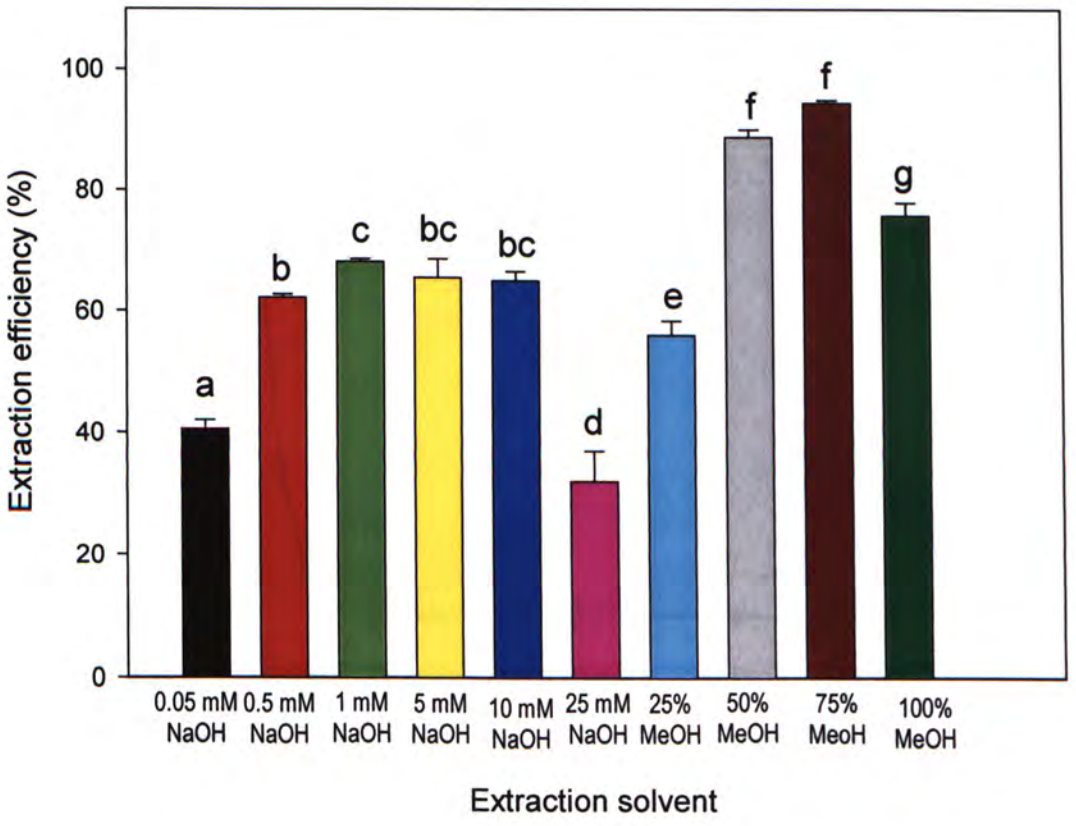

Figure 4.16 The extraction efficiency of PCP from chitin A. Batch experimental conditions: PCP concentration = 100 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g /50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. Extraction experimental conditions: biosorbent concentration = 0.4 g/50 mL of extraction solvent, agitation rate = 200 rpm, temperature = 23±2°C. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

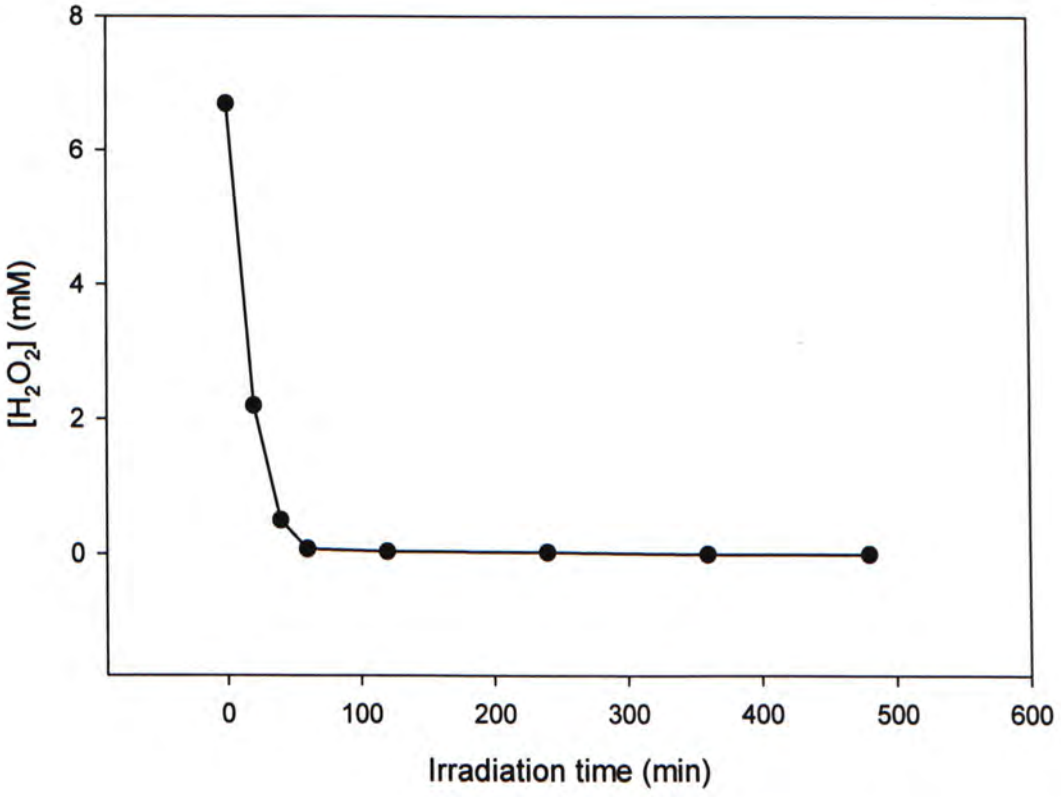

Figure 4.17 The hydrogen peroxide concentration in the batch PCO reaction at different irradiation time monitored by H_2O_2 cell test. Batch experimental conditions: PCP concentration = 100 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.8 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration.

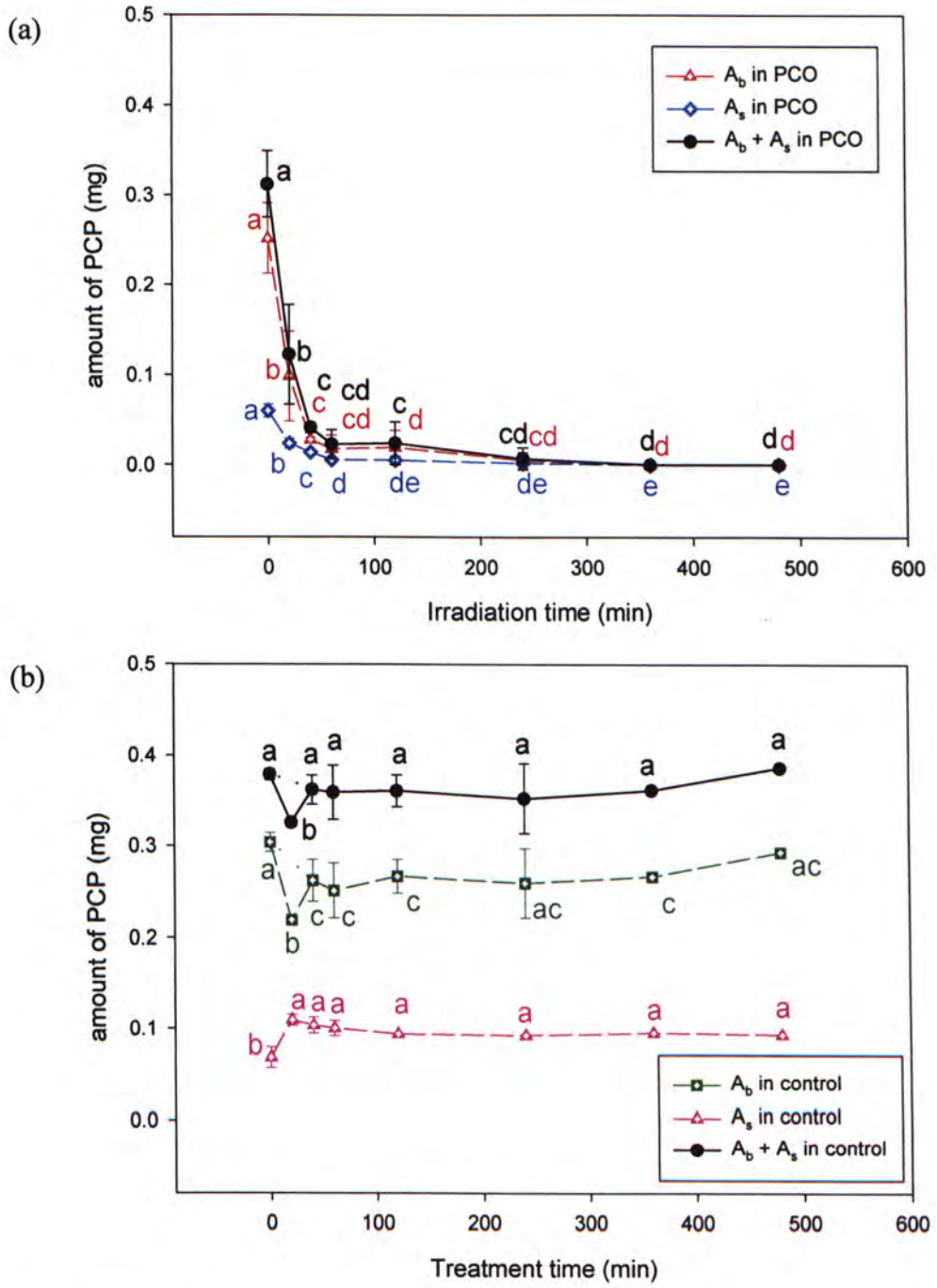

Figure 4.18 The distribution of PCP (a) in PCO and (b) in control with 0.4 g of chitin A. A_s is the amount of PCP in solution; A_b is the amount of PCP on biosorbents. The dotted line represented the reasonable trend. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

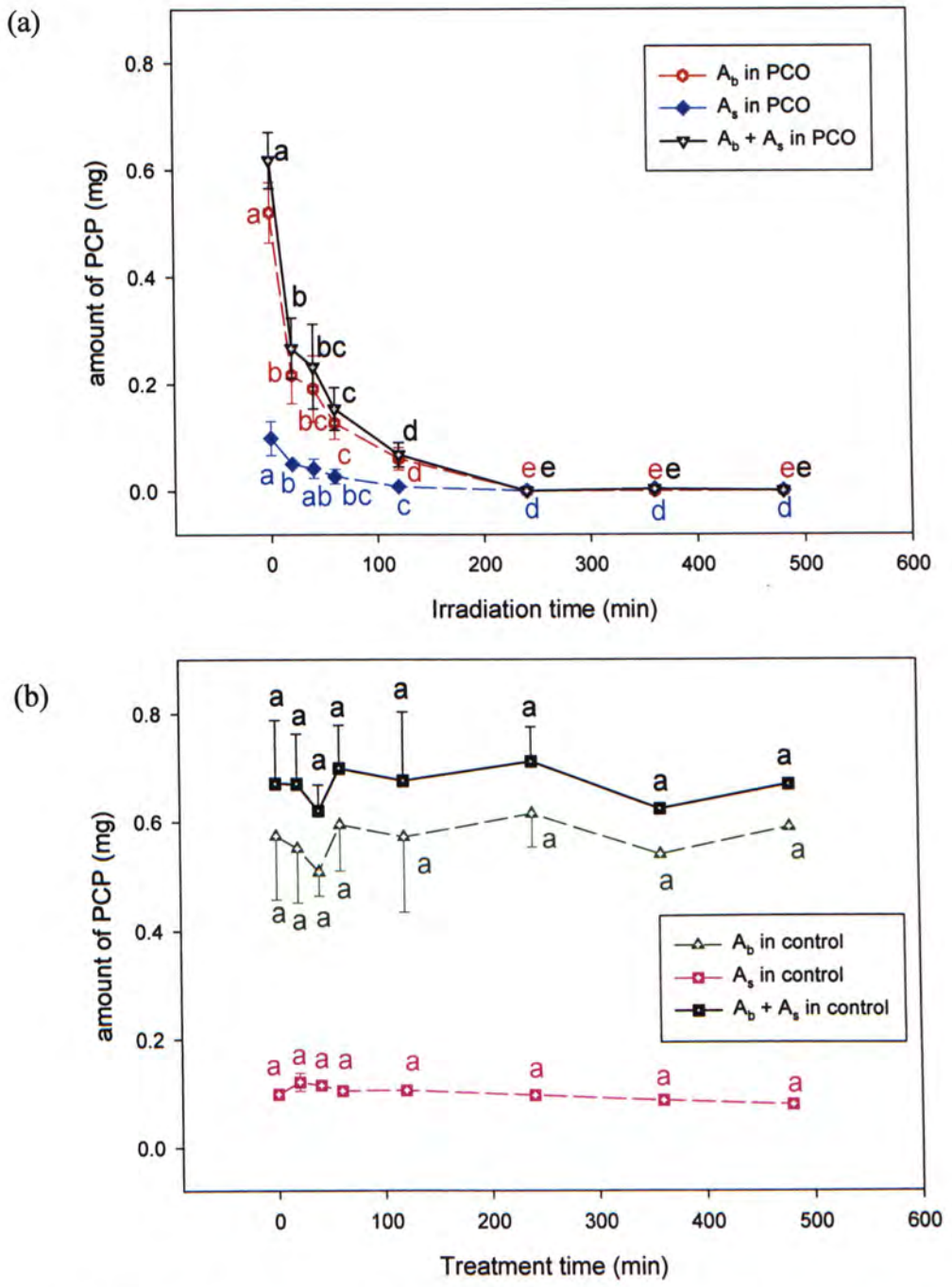

Figure 4.19 The distribution of PCP (a) in PCO and (b) in control with 0.8 g of chitin A. A_s is the amount of PCP in solution; A_b is the amount of PCP on biosorbents. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

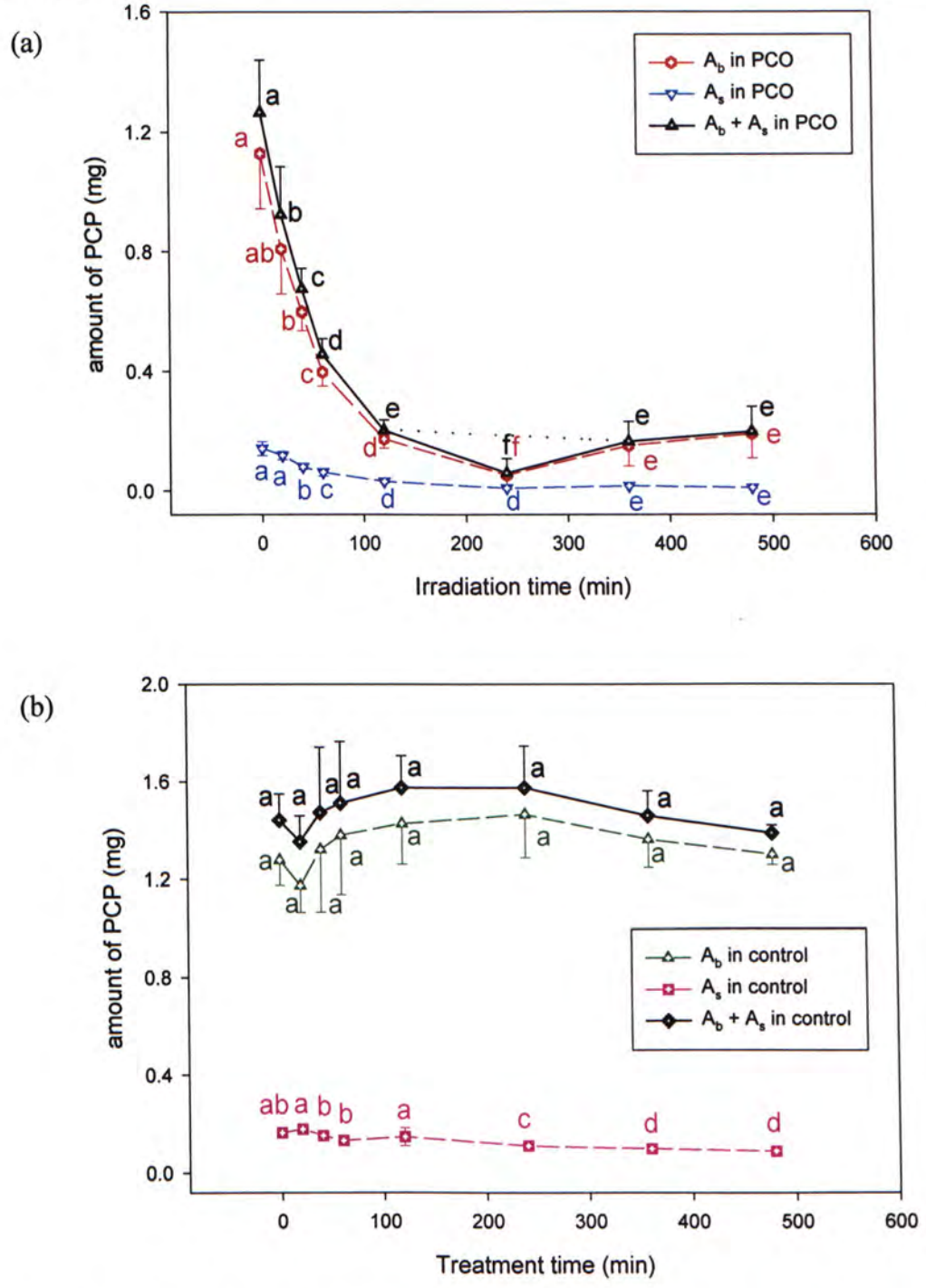

Figure 4.20 The distribution of PCP (a) in PCO and (b) in control with 1.6 g of chitin A. A_s is the amount of PCP in solution; A_b is the amount of PCP on biosorbents. The dotted line represented the reasonable trend. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

less PCP amount was available in the system, small proportional movement resulted in obvious observation. It was noticed that PCP diffused from biosorbent to aqueous medium (A_s) at the first 20 min. After that apparent-equilibrium was made. In addition, it also pointed out that the total amount of PCP in the control system was constant, no PCP lost to the environment (Figures 4.18b, 4.19b and 4.20b).

When considering the distribution of PCP in reaction mixture solution for PCO (Figures 4.18a, 4.19a and 4.20a), it seemed that the total PCP amount ($A_b + A_s$) was always contributed by PCP on biosorbent (A_b). PCP in solution declined continuously until 60 min and reached plateau at very low concentration around 0 mg at 240 min (Figures 4.18a and 4.19a) or 360 min (Figure 4.20a). On the contrary, trends of A_b and $A_b + A_s$ were similar with that of A_s, except that the initial drop was much more sharply.

Figure 4.21 showed the degradation efficiency (DE) of PCP on different biosorbent concentrations for PCO and control. It appeared that the trend of DE for all 0.4, 0.8 and 0.16 g of biosorbents were similar. DE raised rapidly at first 60 min and gradually until 4 h, which nearly 100% DE could be obtained. The only difference but not obvious was that the rate of DE increased at the first 60 min descended from 0.4 g, 0.8 g and 1.6 g. When considering DE of control systems, DE remained at nearly zero for all three biosorbent concentrations. It indicated that all PCP disappeared solely due to reaction of PCO, but not retained on the containers or lost during transfer.

The degradation capacity (DC) of PCP on three biosorbent concentrations, 0.4, 0.8 and 1.6 g, for PCO and control was presented in Figure 4.22. The pattern was the same as that of Figure 4.21. The maximum point of DC (around 0.85 mg of PCP/g of biosorbent at 4 h) was meant all PCP on the biosorbent was degraded, and vice versa for the control system, which nearly no PCP was degraded on the biosorbent.

4.2.4 Effect of PCP amount on biosorbent in PCO

To investigate the effect of PCO to different amount of PCP on biosorbents, 0.4 and 0.8 g of biosorbents, harvested from 10 mg/L and 100 mg/L of biosorption batches as described in Sections 3.3.3 and 3.3.4, were treated by PCO for 60 min under the same conditions. And the results were expressed in Figure 4.23. It seemed that DE was dependent neither on the PCP concentration (Figure 4.23a) nor

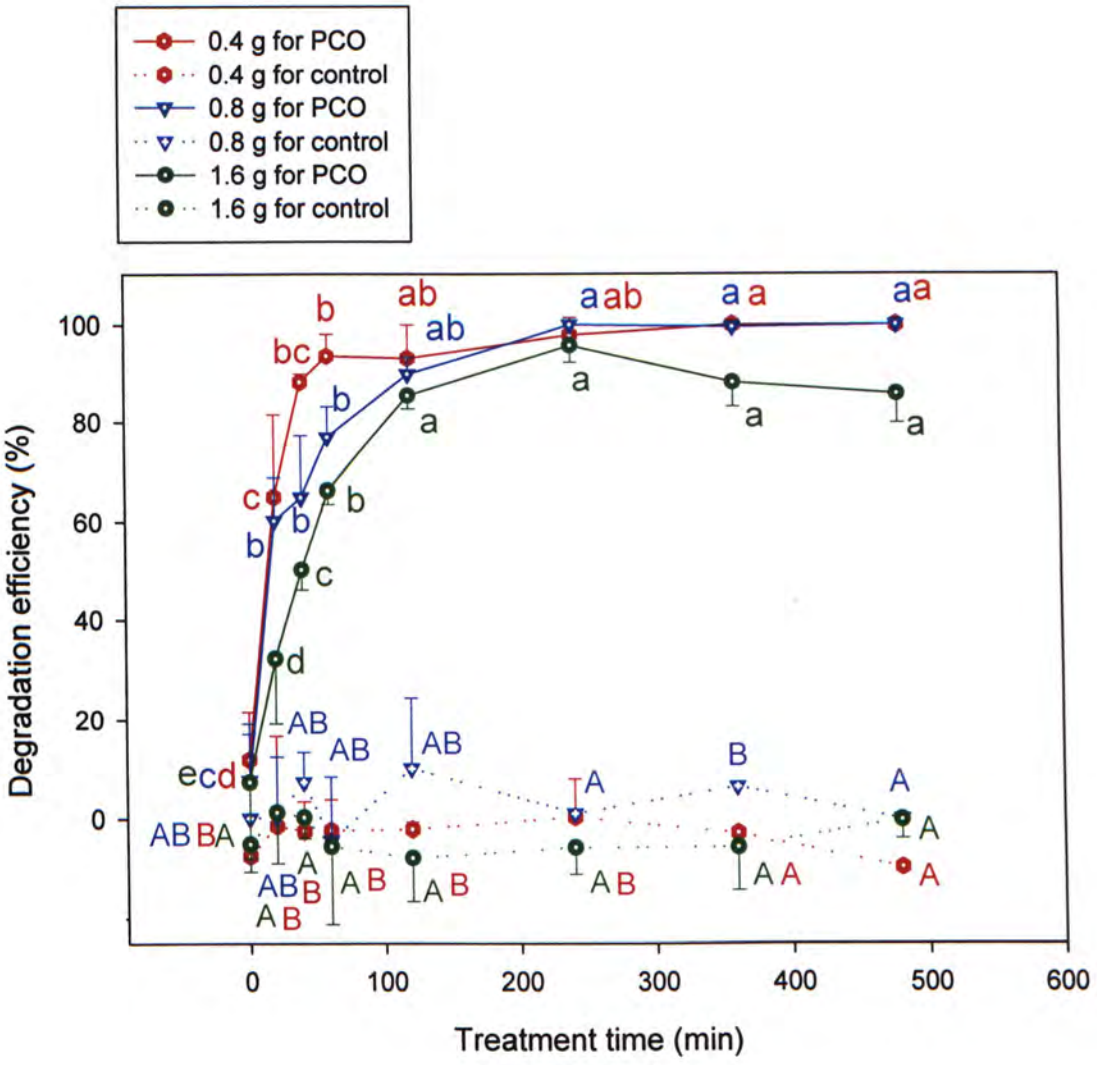

Figure 4.21 The degradation efficiency (DE) of PCP on 0.4, 0.8 and 1.6 g of chitin A by PCO. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4, 0.8 and 1.6 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

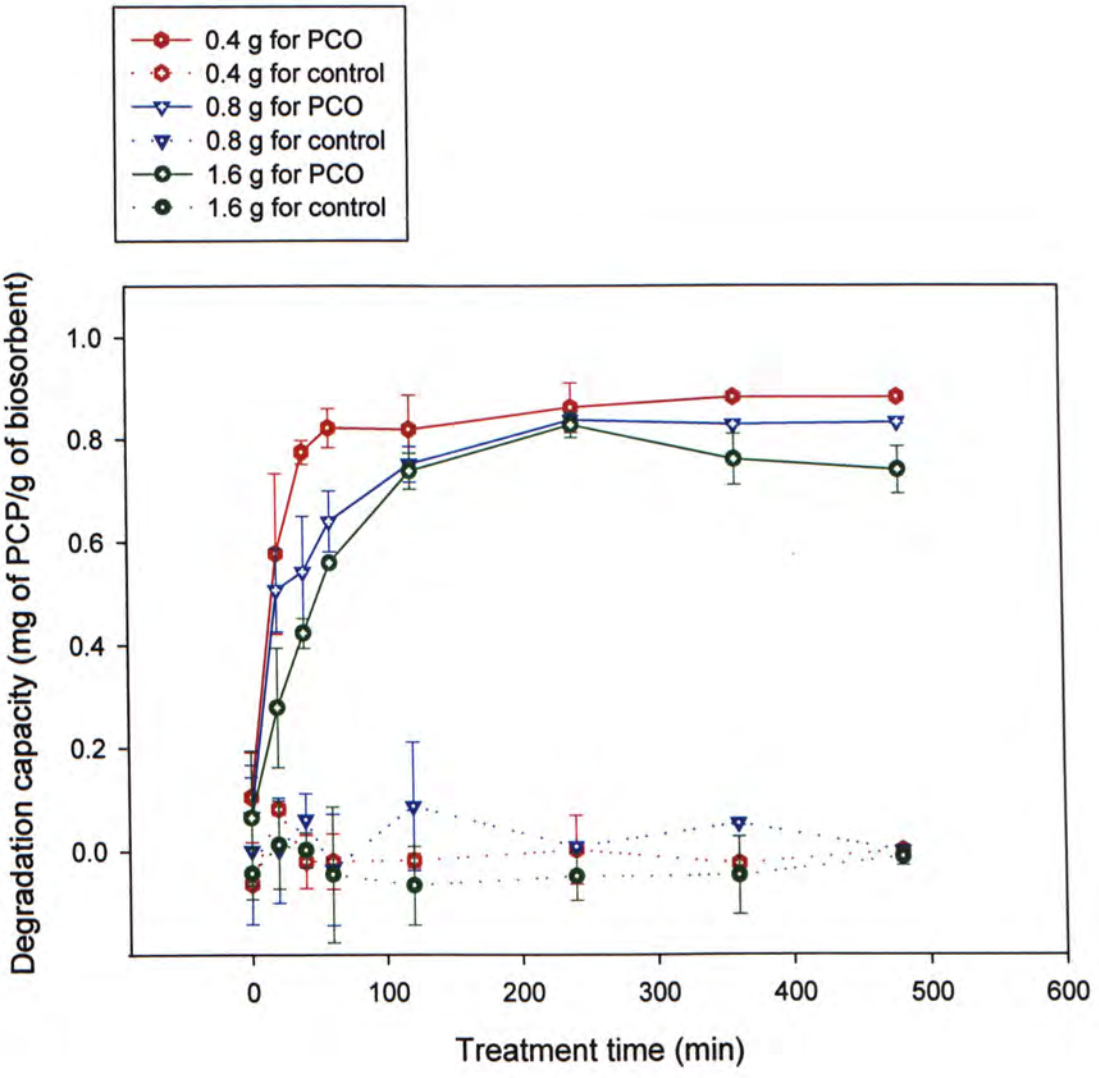

Figure 4.22 The degradation capacity (DC) of PCP on 0.4, 0.8 and 1.6 g of chitin A by PCO. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4, 0.8 and 1.6 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration. The statistical results were the same as that of Figure 4.18.

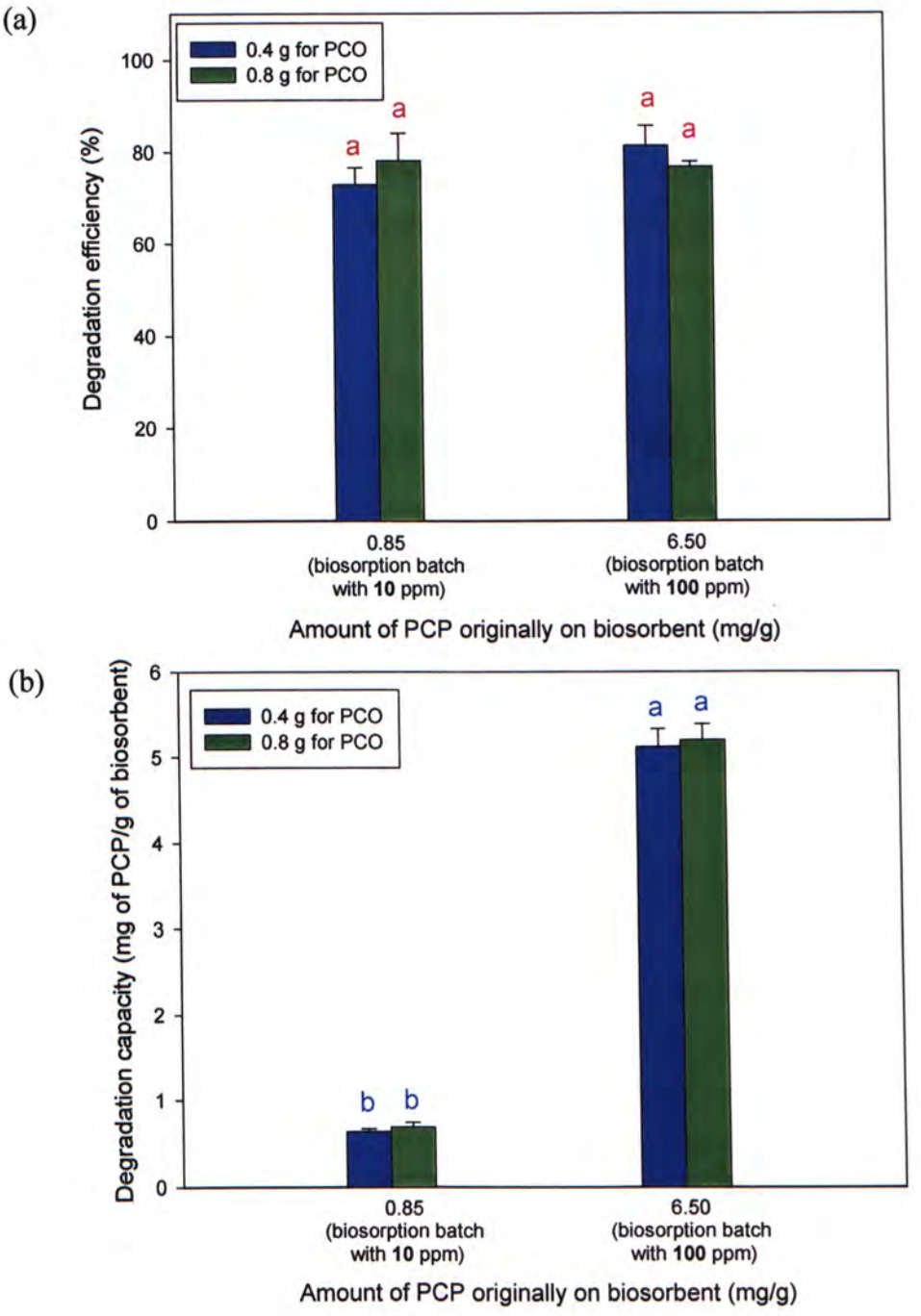

Figure 4.23 The (a) degradation efficiency (DE) and (b) degradation capacity (DC) of different amount of PCP adsorbed on 0.4 and 0.8 of chitin A by PCO, which originally immersed in 50 mL of 10 and 100 mg/L of PCP solutions respectively. Batch experimental conditions: PCP concentration = 10 mg/L and 100 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4 and 0.8 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

biosorbent concentration (Figures 4.21 and 4.23a), as it remained around 80% for the increase of PCP amount on biosorbents (0.8 mg to 6.5 mg) or biosorbent concentration (0.4 g to 0.8 g).

From Figure 4.23b, it was observed that DC for biosorbents with more PCP amount adsorbed (around 6.50 mg of PCP/g of biosorbent) was much higher than that of lower one (approximate 0.85 mg of PCP/g of biosorbent). This phenomenon was expected as higher PCP concentration on biosorbents should yield higher DC with the same DE. On the other hand, it could be concluded that DE was not affected by the removal capacity of PCP on biosorbents.

4.2.5 Determination of chloride ion concentration and total organic carbon during PCO

The degree of dechlorination of PCP by PCO was determined from measuring the chloride ion concentration in reaction mixture (Figure 4.24). The amount of chloride ion increased continuously and reached plateau after 6 h. The amount of Cl^- for 0.8 g of chitin A was nearly doubled of that of 0.4 g. However, the Cl^- amount of 0.8 g of chitin A was not halved of that of 1.6 g. Table 4.3 showed the calculation of the theoretical amount of Cl^- providing that all PCP on biosorbent was dechlorinated. It was noticed that the Cl^- amounts of the experiments at 8 h from all 0.4, 0.8 and 1.6 g of chitin A (Figure 4.24a) were greater than the theoretical amounts. This might be explained as some amount of Cl^- originally existed on the biosorbents. And the same phenomenon was observed in Figure 4.24b. Small amount of Cl^- was detected in the samples of control without prominent increase or decrease.

For total organic carbon (TOC) concentration, the filtrate from 0.4, 0.8 and 1.6 g of chitin A with PCO treatment was analyzed. It appeared that TOC concentration of control (Figure 4.25b) remained approximately constant and the concentration was below 20 mg/L. However, the unexpected results of increasing TOC were observed for PCO treated biosorbent (Figure 4.25a) along the irradiation time. This phenomenon might be attributed to the protein or the other materials released from biosorbent, which masked the results of mineralization of PCP by PCO.

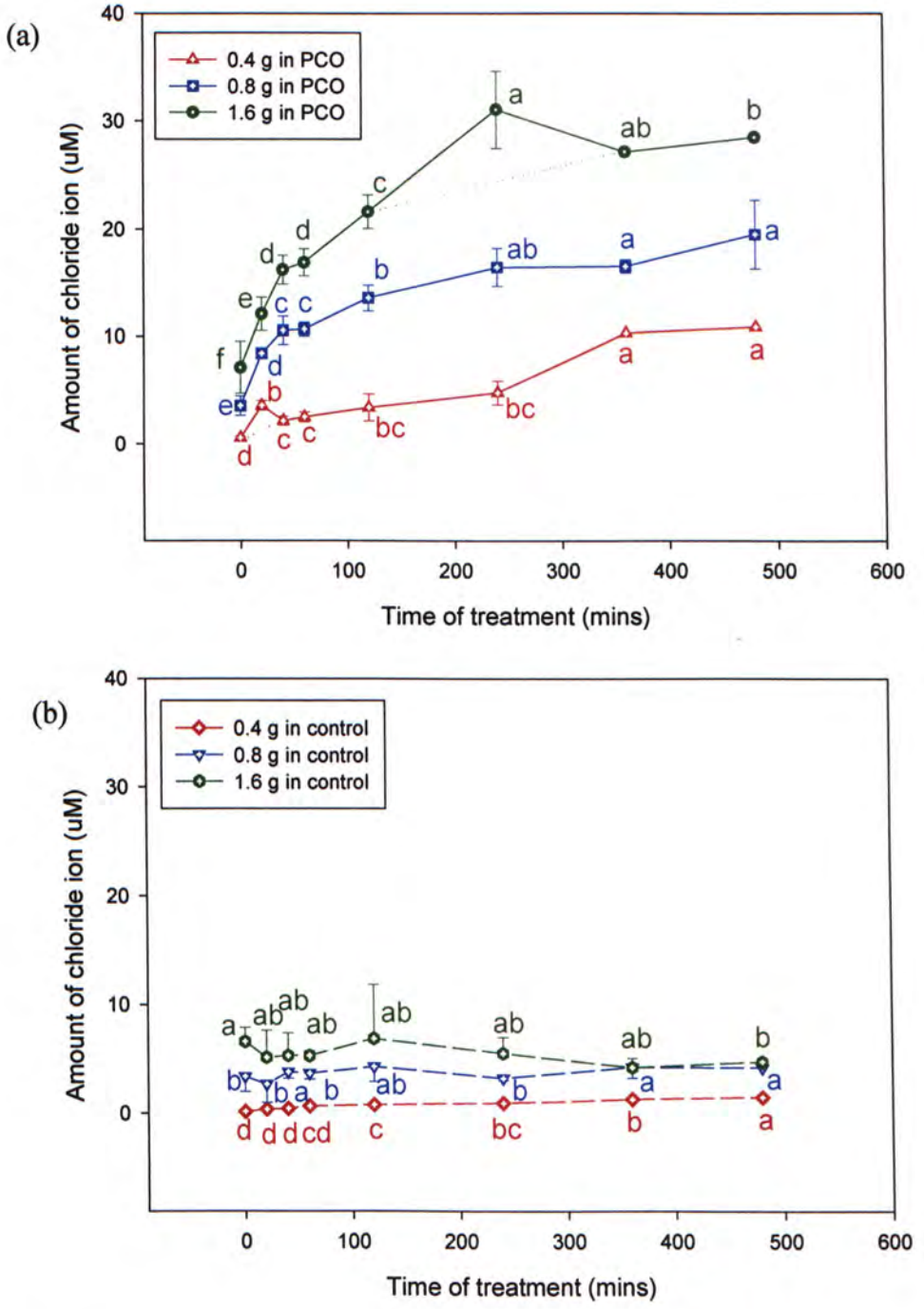

Figure 4.24 The chloride amount in reaction mixture in (a) PCO and (b) control, with 0.4, 0.8 and 1.6 g of chitin A. The dotted line represented the reasonable trend. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4, 0.8 and 1.6 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

Table 4.3 The total amount of chloride ion from PCP adsorbed on chitin A for PCO.

Amount of chitin A (g)	Amount of PCP on chitin A (mg)*	Total amount of Cl^- from PCP on chitin A (μM)**
0.4	0.34	6.39
0.8	0.68	12.78
1.6	1.36	25.57

*The average PCP removal capacity of chitin A when immersing in 50 mL of 10 mg/L of PCP solution under the selected condition is 0.85 mg of PCP/g of biosorbent.

**Molar mass of chloride is 35 g, and 1 M of PCP consists of 5 M of Cl^-.

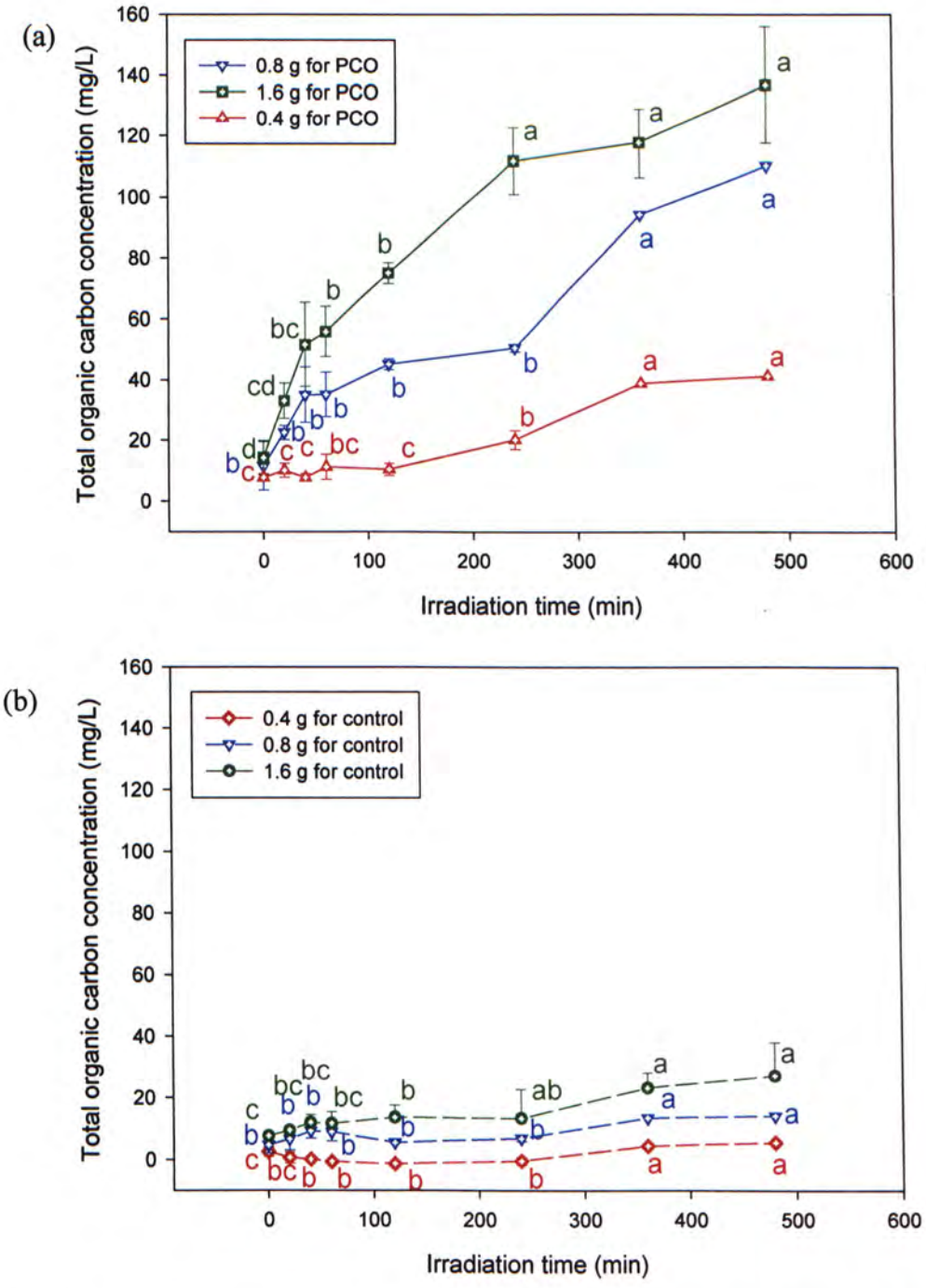

Figure 4.25 The total organic carbon concentration in reaction mixture in (a) PCO and (b) control, with 0.4, 0.8 and 1.6 g of chitin A. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4, 0.8 and 1.6 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

4.2.6 Identification the intermediates of PCP degradation by PCO

The intermediates during the degradation of PCP on biosorbents by PCO were identified and the GC-MS spectrum was shown in Figure 4.26. The most prominent peak (retention time 11.91 min) was the parental compound, PCP. And the intermediates were revealed as 2,3,5,6-tetrachlorophenol ($C_6H_2Cl_4O$) (retention time 9.70 min) and 2,3,5,6-tetrachlorohydroquinone (TeHQ) ($C_6H_2Cl_4O_2$) at 12.08 min.

4.2.7 Evaluation of the change of PCO treated biosorbents

4.2.7.1 Chitin assay

The chitin contents of PCO treated and untreated chitin A and chitin B were tabulated in Table 4.4. Results indicated that chitin B had higher chitin content. And there was no difference in chitin content between the PCO treated and untreated biosorbents.

4.2.7.2 Diffuse reflectance Fourier transform infra-red spectroscopy

Diffuse reflectance Fourier transform infra-red (DRFT-IR) spectroscopy was used to roughly examine any structural change for the untreated and PCO treated biosorbents. Figure 4.27 showed the DRFT-IR spectra of untreated and PCO treated chitin A, chitin B and chitosan. It seemed that the pattern of untreated and PCO treated biosorbents were similar; thus it revealed that the structure of biosorbents after PCO remained at some extend unchanged.

To examine the effect of prolonging PCO to the structural change of chitin A, the structures of chitin A with PCO for 0, 120, 240, 360 and 480 min were analyzed and compared with the untreated one, and the spectrum was shown in Figure 4.28. It was observed that there was no obvious structural change to chitin A for prolonging PCO treatment, as the patterns of the spectrum were all similar.

4.2.7.3 Protein assay

The protein contents of PCO treated and untreated biosorbents, chitin A, chitin B and chitosan, were summarized in Table 4.5. It was obvious that chitin A had the highest protein content, followed by chitin B and chitosan. In addition, it seemed that PCO could degrade some of the protein from the biosorbents.

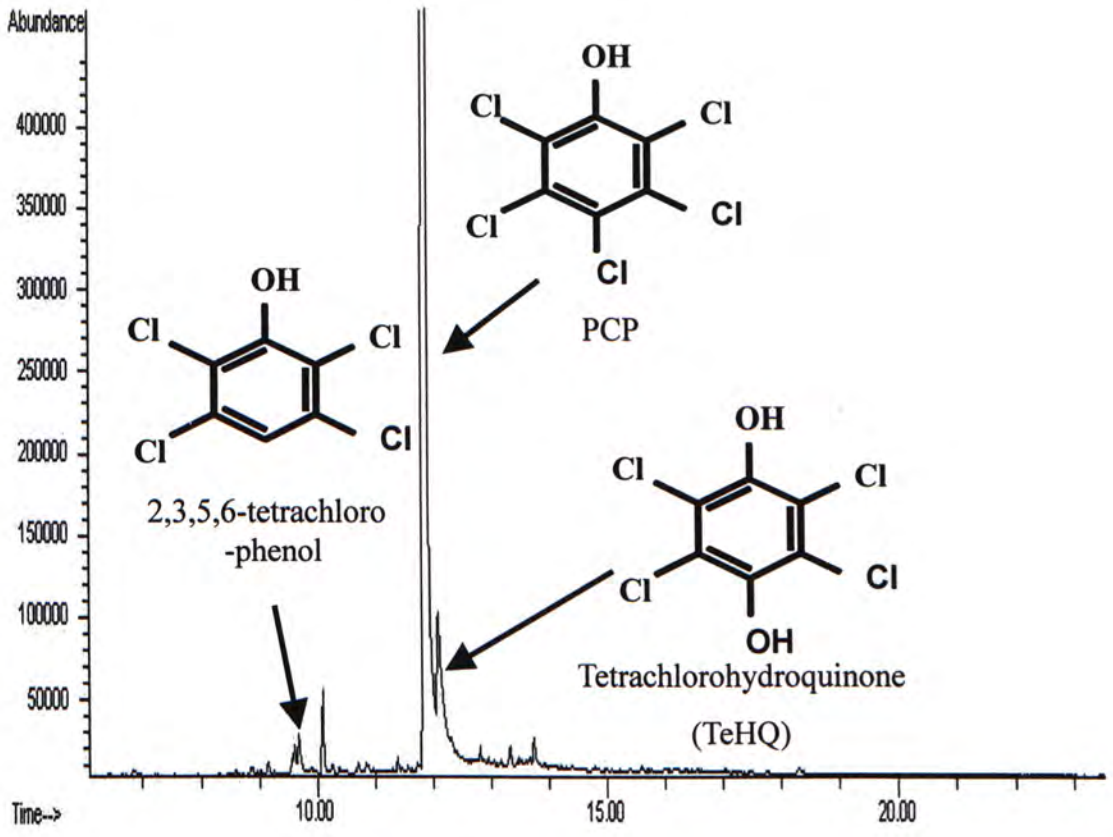

Figure 4.26 GC-MS spectrum of the extract of reaction mixture solution after PCO. Batch experimental conditions: PCP concentration = 100 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.8 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration.

Table 4.4 Chitin contents of PCO treated and untreated chitin A and chitin B.

Biosorbent		Chitin content (%)*
Chitin A	Untreated	71.87 ± 4.63^{a}
	PCO treated	72.90 ± 4.63^{a}
Chitin B	Untreated	91.67 ± 1.96^{b}
	PCO treated	90.38 ± 1.67^{b}

* Means of triplicates with same superscript are statistically identical (One way ANOVA, Tukey, $p < 0.05$) .

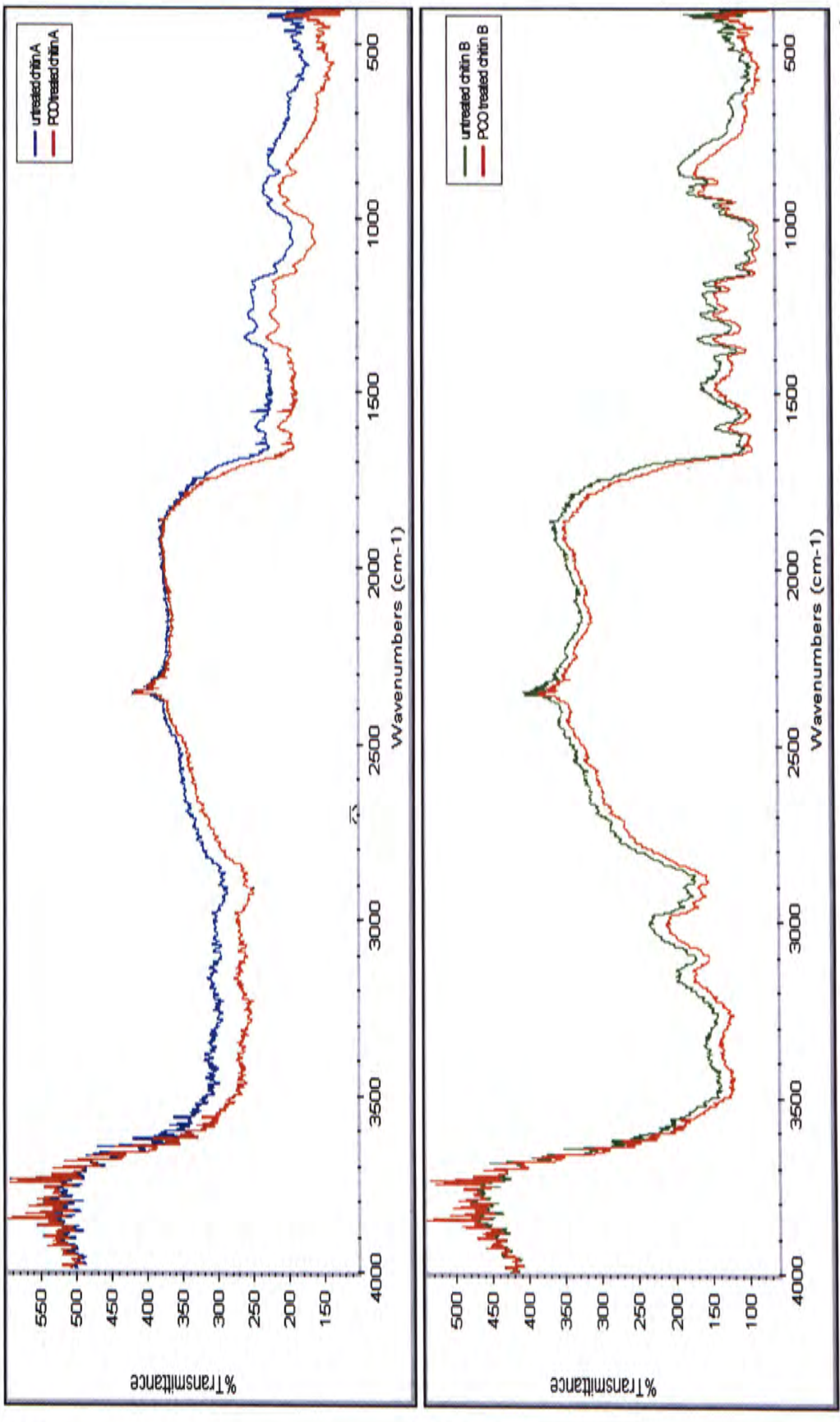

Figure 4.27 Diffuse reflectance Fourier transform infra-red spectra (DRFT-IR) of (a) untreated and PCO treated chitin A, and (b) untreated and PCO treated chitin B. PCO experimental conditions: biosorbent concentration = 0.4 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2, irradiation time = 60 min and reacted with aeration.

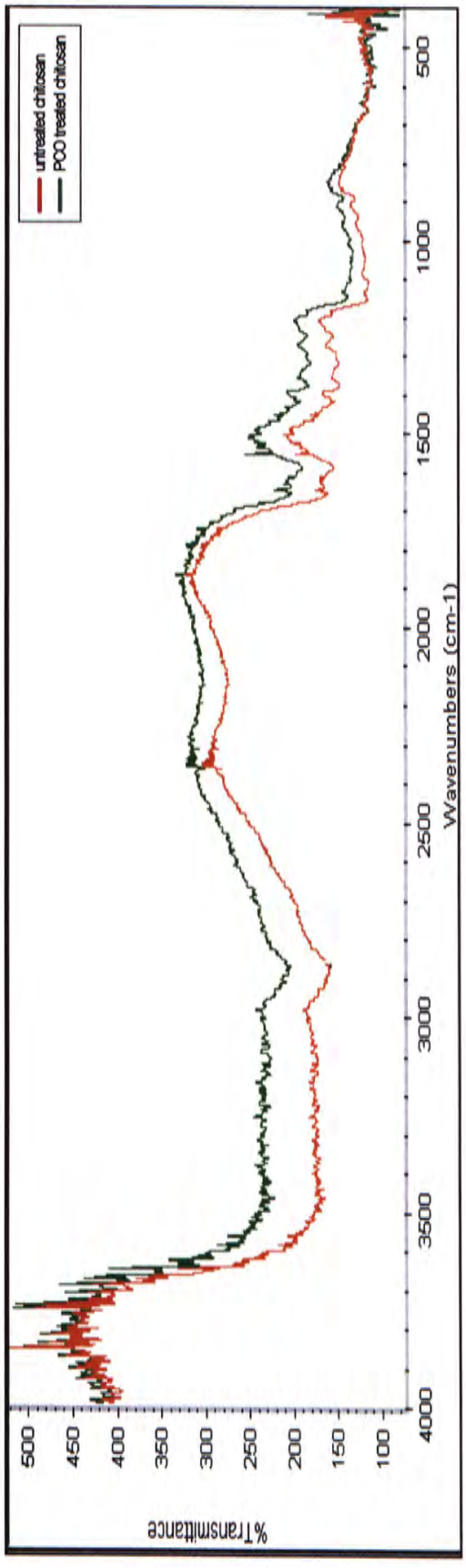

Figure 4.27 Diffuse reflectance Fourier transform infra-red (DRFT-IR) spectra of (c) untreated and PCO treated chitosan. PCO experimental conditions: biosorbent concentration = 0.4 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2, irradiation time = 60 min and reacted with aeration.

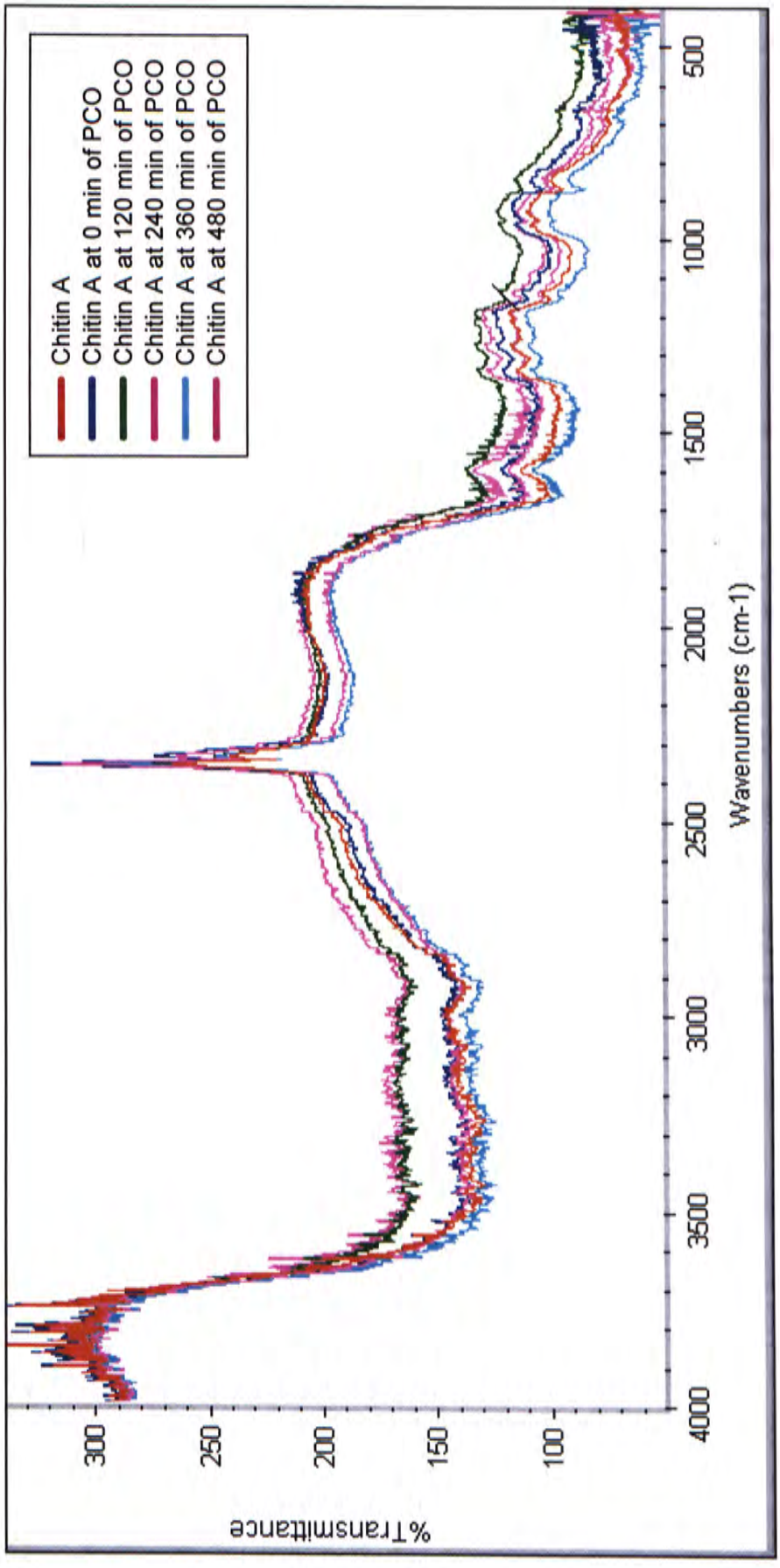

Figure 4.28 Diffuse reflectance Fourier transform infra-red (DRFT-IR) spectra of untreated and 0, 120, 240, 360 and 480 min of PCO treated chitin A. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g /50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2 and reacted with aeration.

Table 4.5 Protein contents of untreated and PCO treated chitin A, chitin B and chitosan.

Biosorbent	Treatment	Protein content (%)*
Chitin A	Untreated	21.09 ± 1.60^{a}
	PCO treated	5.47 ± 2.24^{b}
Chitin B	Untreated	1.12 ± 0.03^{c}
	PCO treated	0.94 ± 0.04^{d}
Chitosan	Untreated	0.73 ± 0.02^{e}
	PCO treated	0.23 ± 0.01^{f}

* Means of triplicates with same superscript are statistically identical (student's t-test).

4.2.7.4 Biosorption efficiency

The removal efficiency of biosorption by untreated and PCO treated chitin A, chitin B and chitosan were compared respectively, in order to examine any deterioration of ability of biosorbents after PCO. The results (Figure 4.29) revealed that PCO caused no difference in biosorption ability of biosorbents, as REs of PCO treated biosorbents were approaching that of untreated. Except that PCO treated chitin A had slightly higher removal efficiency than that of untreated. This might be because TiO_2 itself contributed for adsorption of PCP (Pecchi *et al.*, 2001; Yang *et al.*, 2001).

4.2.8 Multiple biosorption and PCO cycles of PCP

The RE and RC of 0.8 and 1.6 g of chitin A from one, two, three and four multiple biosorption and PCO cycles respectively were compared, so as to investigate the regeneration ability of the biosorbent. From Figures 4.30 and 4.31, both 0.8 and 1.6 g of chitin A from four multiple cycles showed no significant difference for the removal efficiency in adsorbing PCP. It was shown that, after PCO, chitin A had not change in ability even after four multiple cycles, and thus it could be reused again for further biosorption and PCO. In Figure 4.30, RC for 1st biosorption cycle was slightly lower than that of the other three cycles. This might also result from the presence of TiO_2 as it could contribute for PCP adsorption (Zhang *et al.*, 1998; Bissen *et al.*, 2001).

4.2.9 Evaluation for the toxicity change of PCP adsorbed biosorbents during PCO

The toxicity of original PCP was 0.65 (0.61-0.7) and 0.39 mg/L (0.35-0.43) for EC50-5min and EC50-15min of Microtox® test respectively (Fong, 2001). The toxicity change of 0.85 mg/g of PCP adsorbed chitin A (0.4 g of chitin A immersed in 10 mg/L of PCP) during PCO and control was presented in Table 4.6. It showed that the toxicities of control remained constant with approximate 400 and 300 mg/Kg for EC50-5min and EC50-15min respectively. It was reasonable as there was no disappearance of PCP on biosorbent occurred during the experimental time. The toxicity of PCP adsorbed chitin A at first 60 min irradiation time was higher than that of untreated chitin A (1182.47 mg/Kg for EC50-15min) and PCO treated chitin A

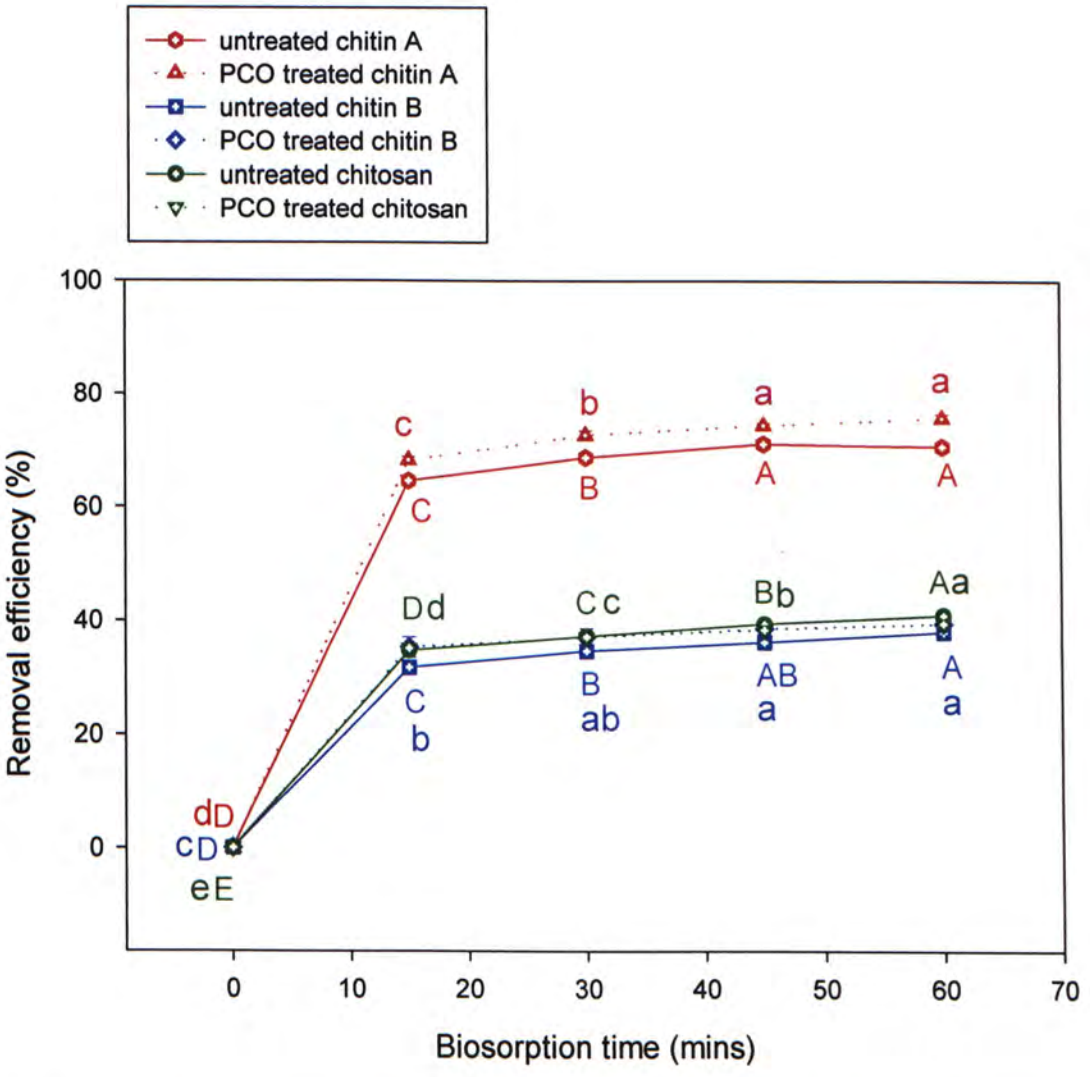

Figure 4.29 The removal efficiency (RE) of PCP on 0.4 g of untreated and PCO treated chitin A, chitin B and chitosan. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.4 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2, irradiation time = 60 min and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$).

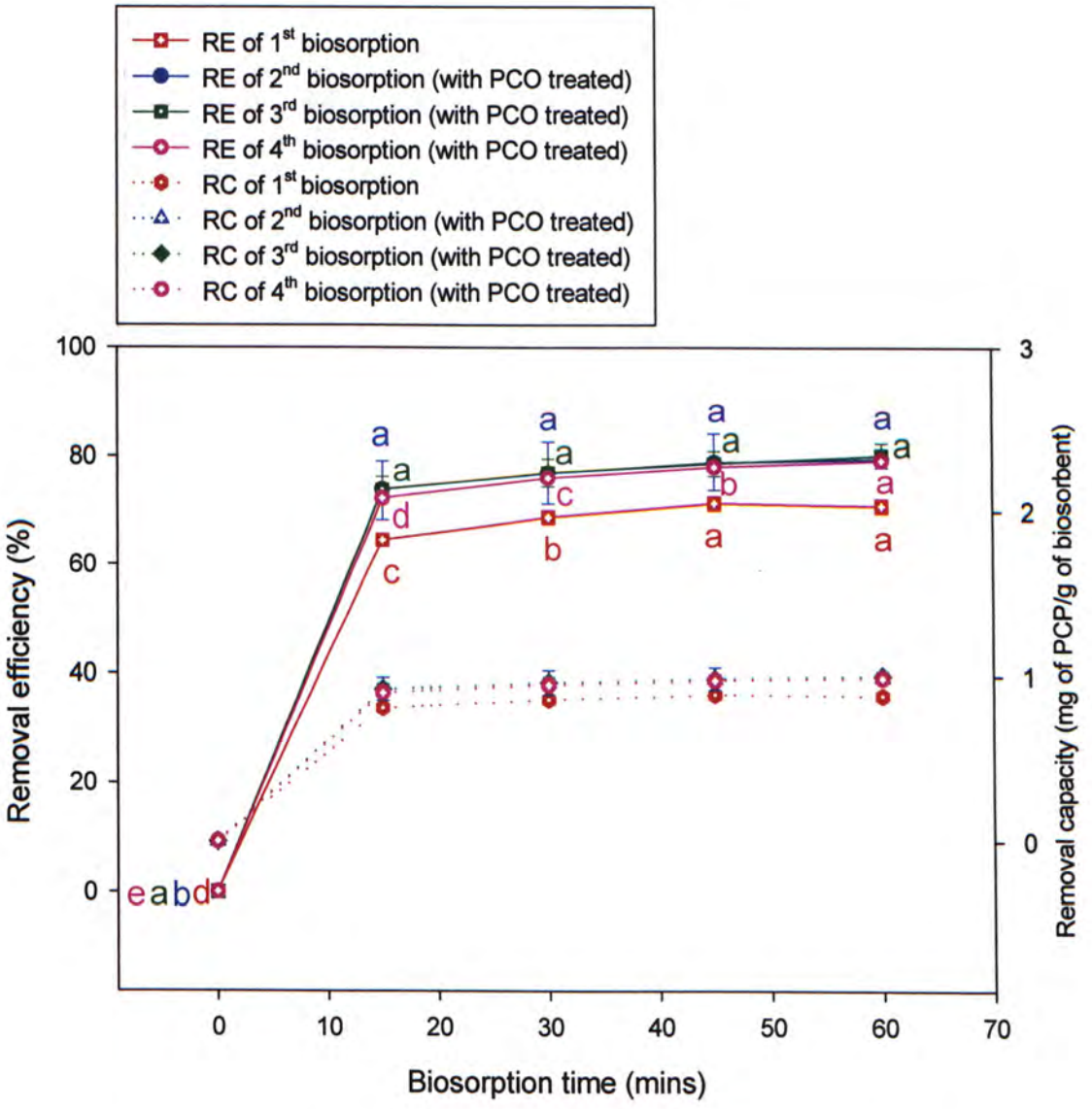

Figure 4.30 The removal efficiency (RE) and removal capacity (RC) of multiple biosorption and PCO cycles of 0.8 g of chitin A. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.8 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2, irradiation time = 240 min and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$). RC had the same statistical results as that of RE.

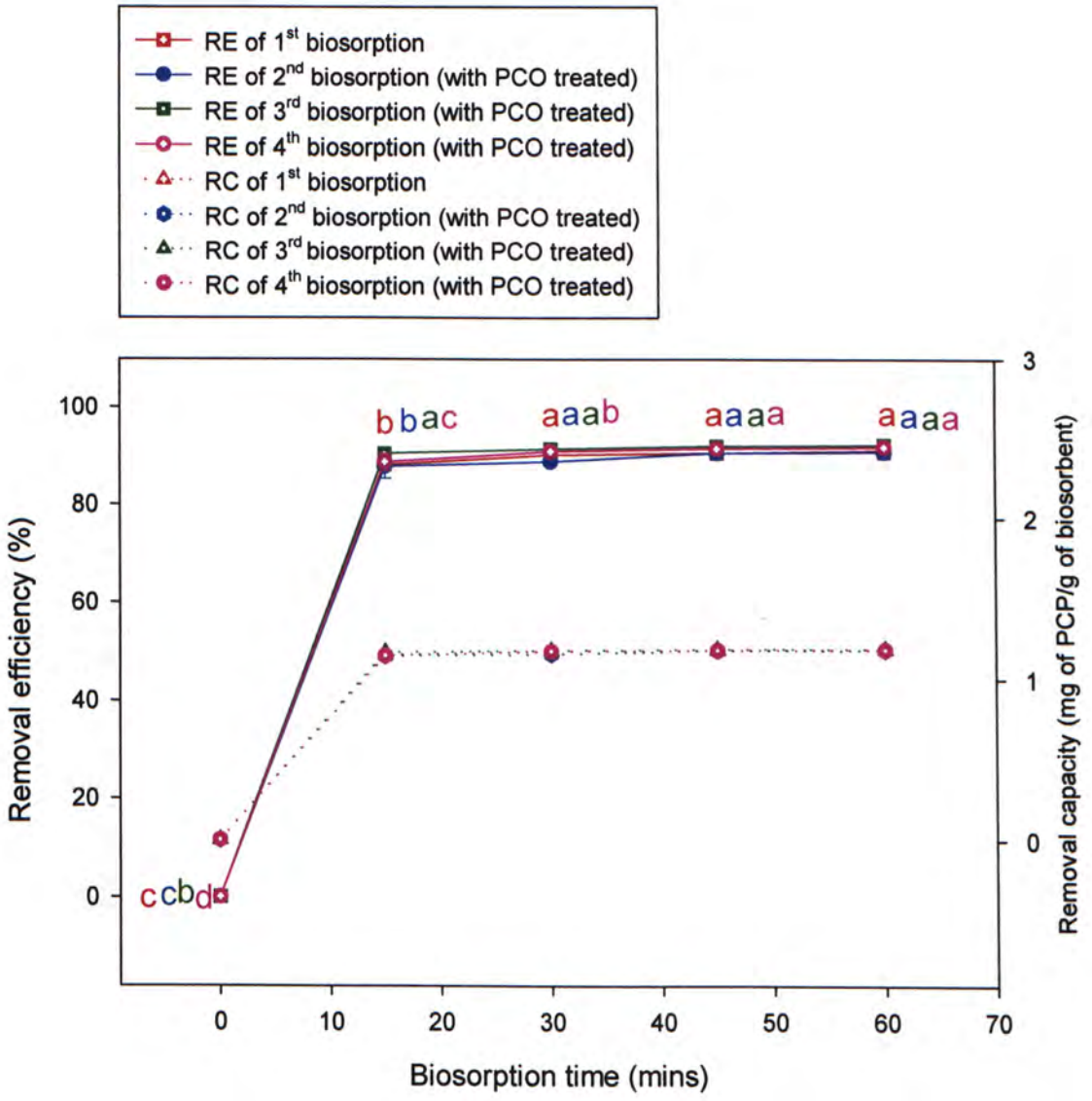

Figure 4.31 The removal efficiency (RE) and removal capacity (RC) of multiple biosorption and PCO cycles of 1.6 g of chitin A. Batch experimental conditions: PCP concentration = 10 mg/L, initial pH = 6.5, biosorbent concentration = 0.4 g/50 mL of PCP solution, agitation rate = 200 rpm, temperature = 23±2°C, contact time = 60 min. PCO experimental conditions: biosorbent concentration = 0.8 g/100 mL of reaction mixture solution, TiO_2 concentration = 200 mg/L, H_2O_2 = 6.7 mM, intensity of eight UV (365 nm) lamps = 33 W/m^2, irradiation time = 240 min and reacted with aeration. Data represent the means and error bars represent the standard deviations of triplicates. Means with the same color and letter are statistically identical (One way ANOVA with Tukey test, $p < 0.05$). RC had the same statistical results as that of RE.

Table 4.6 Toxicity change of 0.8 mg/g of pentachlorophenol on chitin A during PCO for the Microtox® test.

	Irradiation time (min)	EC50-5 min (mg/Kg)	EC50-15 min (mg/Kg)
PCO treated	0	511.14 (338.62-771.57)	488.12 (211.19-1128.19)
	20	766.42 (585.69-1002.92)	815.94 (484.41-1374.37)
	40	581.85 (280.36-1207.57)	602.69 (284.33-1277.51)
	60	675.08 (517.04-881.42)	651.62 (410.23-1035.05)
	120	1282.30 (501.39-3279.51)	1214.93 (475.26-3105.76)
	240	2616.14 (1437.35-4761.66)	2400.73 (1434.04-4019.06)
	360	2766.47 (2283.71-3351.30)	2837.12 (2222.70-3621.39)
	480	4595.58 (643.60-5796.30)	3771.80 (3153.44-4511.41)
Control	0	331.36 (117.22-936.69)	295.98 (148.36-590.49)
	20	374.80 (215.47-651.95)	331.94 (170.07-647.89)
	40	439.62 (259.13-745.81)	383.03 (243.25-603.14)
	60	262.65 (243.63-283.15)	249.62 (204.21-305.12)
	120	318.49 (146.24-693.66)	232.93 (95.21-569.84)
	240	529.81 (368.49-501.32)	305.90 (236.66-395.40)
	360	690.27 (429.40-1109.64)	508.09 (286.32-901.66)
	480	456.25 (352.23-591.00)	361.97 (235.92-555.37)
Untreated chitin A		1050.98 (669.66-1649.45)	1182.47 (703.45-1987.70)
PCO treated chitin A		1325.61 (858.98-2045.74)	908.71 (263.13-3138.17)

Data in parenthesis represented the 95% confidence range.

(908.71 mg/Kg for EC50-15min), as PCP on biosorbent mainly contributed to the toxicity. And the toxicity declined along the irradiation time as PCP was degraded by PCO. When the PCP adsorbed chitin A was treated with PCO for more than 2 h, most of PCP on biosorbent was degraded (as in Figures 4.21 and 4.22). Thus the toxicity of it was just similar with that of untreated chitin A. For prolonged PCO treatment, some toxic materials from chitin A was also degraded and thus progressing decreased in toxicity as result.

5. Discussion

5.1 Batch biosorption experiment

5.1.1 Selection of optimal conditions for batch PCP adsorption

It is well documented that the physico-chemical conditions of sorbate-sorbent system highly influence the performance of biosorption (Jacobsen *et al.*, 1996; Ning *et al.*, 1999; Jianlong *et al.*, 2000; Sag & Kutsal, 2000). And some physico-chemical parameters can give an insight into the mechanism of biosorption (Nelson & Yang, 1995; Bousher *et al.*, 1997; Viraraghavan & Slough, 1999). Therefore, to realize the effect brought from changing experimental conditions is of great importance. In addition, it can give a reference to select a condition yielding better biosorption performance.

5.1.1.1 Effect of initial pH

Based on the results obtained, solution pH showed significant effects on the PCP removal. Generally, the removal efficiency increased with declining pH (Figures 4.1, 4.2, 4.3 and 4.4). These results are in good agreement with many previous studies investigating the effect of PCP adsorbed by adsorbents such as soils, powdered or granular activated carbon, peat-bentonite mixtures and activated sludge biomass (Christodoulatos & Mohiuddin, 1996; Mollah & Robinson, 1996a; DiVincenzo & Sparks, 1997; Kjøniksen *et al.*, 1997; Piron *et al.*, 1997; Hu *et al.*, 1998; Viraraghavan & Slugh, 1999; Jianlong *et al.*, 2000).

As mentioned in Section 1.2.2.1, since PCP has low dissociation constant, pK_a (= 4.74), the neutral form is dominant at low pH (< 4.74). And this neutral form favors the adsorption by organic sorbents, which can be expressed by high log P_{ow}(HA) (= 5.1). However, in neutral or alkaline conditions, 99% of PCP appeared as ionized form and thus the adsorption is reduced given that log $P_{ow}(A^-)$ is 1.5. Based on this speculation, the removal efficiency (RE) is increased with decreasing pH. And it can induce that the major adsorption mechanism should be due to the interaction between hydrophobic non-ionizable organic compounds (HNOCs) such as hydrophobic force, Van der Waals forces, or hydrogen bonding (Nelson & Yang, 1995; You & Liu, 1996; Bousher *et al.*, 1997; Viraraghavan & Slough, 1999). It is worth to note that the PCP RE from chitin B increased sharply at pH 2.5, even higher than that of chitosan. This phenomenon also supports the proposed mechanism of

adsorption. At low pH, the hydroxyl (OH) functional group of protonated PCP can form hydrogen bonding with carbonyl (—C=O) of chitin (Figure 5.1) (Nelson & Yang, 1995; Viraraghavan & Slough, 1999). This largely enhances the adsorption attributed to the increase proportion of neutral PCP. However, providing that the intrinsic pK_a of amine functions ($-NH_3^+$) of chitosan is close to 6.5 (as expressed in Section 1.2.2.1), the chitosan exhibits positively charged with the availability of H^+ under acidic condition ($pH < 6.5$). This electrostatic property does not favor the adsorption of neutral PCP. Meanwhile, at higher pH ($4.74 < pH < 6.5$), this electrostatic property of chitosan might be responsible for the adsorption of ionized PCP (Onsøyen & Skaugrud, 1990). And thus it gave higher RE than chitin B at higher pH. In addition, with the pH increases, both PCP and the overall surface charge of biosorbents become more and more negative and therefore the adsorption is limited. Meanwhile, the mechanism of PCP adsorption on chitin and chitosan might involve not only the mentioned electrostatic force and hydrogen bonding. It should be very complicate with considering the proportion of ionized or neutral sorbent as well as sorbate, the effect of other environmental factors and the impurity of the biosorbents themselves.

By considering the real situation, the pH range of natural systems containing PCP is usually at pH 5-8 (Christodoulatos & Mohiuddin, 1996). And it is not environmental friendly and cost-effective to adjust the solution to extremely low pH with the expense of large amount of strong acid. Therefore, pH 6.5 was chosen in later experiment as it showed biosorption performance.

When considering the efficiency among the three biosorbents, chitin A had the highest RE, followed by chitosan and chitin B (Figure 4.4). The same result was obtained from previous study that crude chitin had the highest efficiency for metal ions removal compared with the pure chitin and chitosan (Tsui, 2000). This should be attributed to the particle size, their compositional difference and the degree of depolymerization. It is noted that the particle of chitin A is irregular and relatively smaller (Plate 3.2a). Whereas chitin B and chitosan have similar outlook with uniform sheet-form and the particles are larger compared with chitin A. Therefore, chitin A might favour the adsorption as for the larger surface area available for the PCP adsorption.

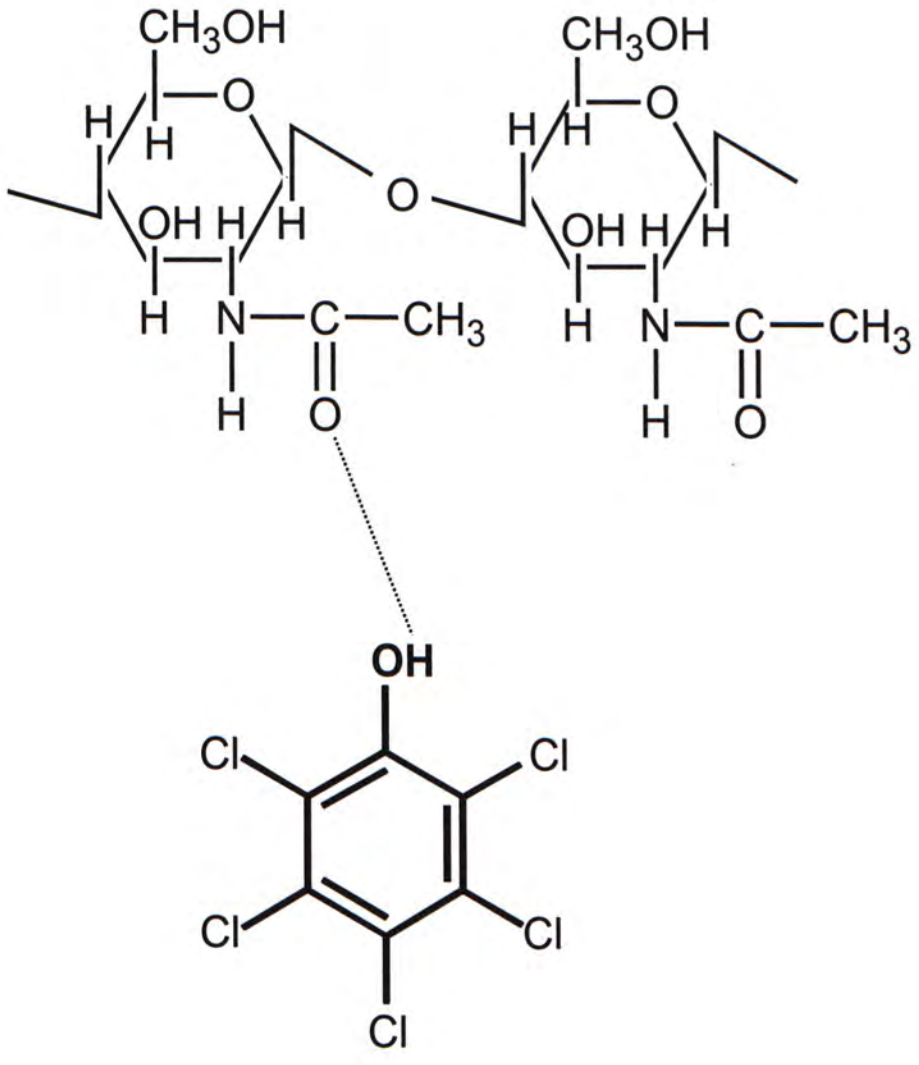

Figure 5.1 The hydrogen bonding (·····) formed between carbonyl group of chitin and hydroxyl group of PCP (Nelson & Yang, 1995; Viraraghavan & Slough, 1999).

Besides particle size, the composition of biosorbents as well as the degree of *N*-deacetylation (DDA) influences the performance of biosorption. DDA is the ratio of D-glucosamine to *N*-acetyl-D-glucosamine structural units. That is, it is a measure to quantify the acetyl groups (—$COCH_3$) removed during the acetylation process and leaving the amino groups (—NH—) on the structure (Onsøyen & Skaugrud, 1990; Muzzarelli *et al.*, 1994; Cho *et al.*, 1998; Tsui, 2000). As aforementioned (Section 4.1.1.1), the presence of free amine group favors the adsorption owing to electrostatic forces. In theory, chitosan has the highest DDA and thus it gives the highest RE based on this postulated principle. However, chitosan only showed higher RE than chitin B, but not chitin A. It indicates that there is another factor apart from DDA determining the performance. It is observed that the major difference of chitin A and chitin B is the composition. Chitin B is pure chitin with chitin content more than 91% (from Table 4.4) obtained after the process of demineralization (described in Section 1.2.1.3). Meanwhile, Chitin A is the crude chitin obtained directly from grounding the washed shrimp shells. Other than chitin (72%, from Table 4.4), the biosorbent still consists of different components including protein (21%, from Table 4.5), calcium and magnesium carbonate and possibly small amount of lipids as mentioned in Section 1.2.1.3. These impurities might also contribute to the adsorption.

Last but not the least, the occurrence of depolymerization of chitin B and chitosan during the production processes might influence the biosorption performance (Muzzarelli, 1997; Tsui, 2000). As demonstrated in Section 1.2.1.3, the production of chitin B and chitosan requires a series of treatment process involving demineralization and deproteination with acid and base solution, and deacetylation under the high temperature and strong alkaline condition. This harsh depolymerization process is accomplished by cleavage of glycosidic linkages (Shimahara *et al.*, 1984; Tsui, 2000). This can affect the PCP adsorption ability of biosorbent. And thus lower PCP RE of chitin B and chitosan are resulted.

5.1.1.2 Effect of Tris buffer and biosorbent concentrations

In the previous section, it was observed that the PCP RE of biosorbents at extremely low pH was higher. However, it was also noted that the final pH of the batch experiment changed and kept within a narrow constant range (from 8.1 to 9.7

for chitin A; from 7.0 to 8.2 for chitin B; and from 7.6 to 8.6 for chitosan) (Figure 4.4). That was, the pHs of the solutions changed continuously during the batch experiment until reaching a buffering range of biosorbents. Since the PCP biosorption is significantly influenced by solution pH, it is necessary to determine the effect under the constant pH condition. With the presence of Tris buffer to keep the solution pH at 6.5, it enhanced the RE for 22% (Figure 4.6) compared with that of the non-buffering batch system (Figure 4.4). Therefore, it is realized that keeping the conditions at constant pH can yield higher efficiency. However, in the present study, biosorption is applied for economic treatment for the emergence PCP spillage or preconcentration of wastewater (as described in Section 1.1.5.4). It is unusual and not economical to apply buffer solution before biosorption. But it gives valuable information on the importance effect of pH.

Besides solution pH, the biosorbent concentration is another important parameter that influences the biosorption performance (You & Liu, 1996; Sag & Kutsal, 2000). It was found that PCP RE increased with biosorbent concentration (Figures 4.1, 4.2, 4.3, 4.6, 4.7 and 4.8), as more binding sites were provided for sorbates. But the RC, unlike the RE, increased with the decrease of biosorption concentration (Figures 4.6, 4.7 and 4.8). That meant more adsorbates would be adsorbed per unit gram of biosorbent at low concentration of biosorbent than at high concentration of biosorbent. This result induced that high concentration of biosorbent could make a screening effect on the outer layer of biosorbents, and hence protect the binding sites form sorbates (Pons & Fuste, 1993; Chu, 2000). Therefore, the biosorbent concentration was standardized as 0.4 g because its fair performance gave rooms for obvious improvement while experimental changing the other environmental conditions in the later experiments. Such design would balance the effect of both RE and RC.

5.1.1.3 Retention time

As introduced in Section 1.2.2.3, the process of biosorption is time dependent and generally characterized as biphasic process, an initial rapid step followed by a slow step. From the kinetic experiments (Figures 4.1, 4.2, 4.3, 4.6, 4.7 and 4.8), a typical initial rapid step was found consistent with many previous studies (DiVincenzo & Sparks, 1997; Aksu, 1998; Slaney & Bhamidimarri, 1998; Guibal *et al.*, 1999; Sag & Kutsal, 2000). In the first 15 min, the PCP adsorption was very fast.

For biosorption on 0.4 g of chitin A at initial pH 6.5, 64% of PCP was adsorbed (Figure 4.1). This might be contributed to the sufficient availability of binding sites on the biosorbent for PCP adsorption. But after that, the adsorption slowed down until 60 min with RE 71%. This might be the on-set of the second slow step as the PCP removal was less than 5% when prolonging time to 120 min (data not shown), though the biphasic process was not a clear-cut step. And this slow step might be attributed to the steric hindrance between the adsorbed and unadsorbed sorbates, as the occupancy of sorbates on the binding sites could result in reducing the driving force to transport the sorbate to the binding sites. In addition, it is also probably due to the interparticle diffusion of sorbate into the biosorbent (see Section 1.2.2.3) (Lau, 2000; Sag & Kutsal, 2000). According to the principle defined by USEPA that the equilibrium is the minimum amount of time required to establish a change of less than 5% of solute concentration (Viraraghavan & Slough, 1999), 60 min in this study is so-called equilibrium. However, there is no evidence to point out less than 5% PCP removal when lengthening the retention time to a few days, in which the true equilibrium time is usually found. Therefore, in my present study, 60 min can be solely named as apparent-equilibrium (DiVincenzo & Sparks, 1997).

From the industrial point of view, the rapid adsorption kinetic is more practical and desirable in use, as the wastewater containing PCP should be treated efficiently and economically with a short period (Tsui, 2000). It is not recommended to prolong the contact time in order to yield less than 5% of PCP removal. Therefore, 60 min was chosen as the optimum equilibrium time for all the following experiments.

5.1.1.4 Effect of temperature

Based on the results, the PCP adsorption on chitinous materials decreased with high temperature (Figure 4.9). With reference of the information in Section 1.2.2.4, the temperature is the main effect on the chemical bonding between PCP molecule and biosorbents, and it overcomes the influence on ionization constants of PCP (Mehrian *et al.*, 1991; You & Liu, 1996; Mobed & Chang, 1998; DiVincenzo & Sparks, 2001). This result indicates that the adsorption process might be exothermic, which requires a specific surface functional group for bonding, e.g. hydrogen bond formation or charge transfer. With considering the speculation in Section 5.1.1.1, the mechanism of PCP adsorption on chitinous materials in my study might be due to

hydrogen bonding between the carbonyl group of chitin and hydroxyl group of PCP. By the way, the mechanism should be very complicate and involve not only one mechanism. This proposed mechanism can only provide a plausible explanation for the complex reactions between PCP and the surface chitinous materials (Nelson & Yang, 1995). In addition, high temperature favour desorption. And this also can account for the decrease PCP adsorption on biosorbent with increasing temperature.

In practically use, the biosorption taken under room temperature ($23 \pm 2^{\circ}C$) is the most economic and feasible, and it gives additional benefit with higher efficiency. Therefore, it was selected as the optimal condition in all following experiments.

5.1.1.5 Effect of agitation rate

Agitation is a way to facilitate the contact between the sorbate and sorbent. The increase of shaking rate advanced the biosorption (Figure 4.10). With considering the economic point of view, 200 rpm was chosen as one of the optimal conditions, since it was not worth to increase the agitation rate into 300 rpm in order to obtain less than 5% increase of PCP removal.

5.1.2 Effect of initial PCP concentration and biosorbent concentration

In the batch isotherm studies, all biosorbents showed higher PCP RC with increasing initial PCP concentration (Figures 4.11, 4.12 and 4.13). This might be due to a higher probability of contact between sorbates and sorbents as mentioned in Section 1.2.2.6. For chitin B and chitosan, the biosorption phenomenon was consistent with many previous studies with RC steeply increased at low sorbate concentration, but reached plateau at high sorbate concentration (Figures 4.12 and 4.13) (Nelson & Yang, 1995; DiVincenzo & Sparks, 1997; Guibal *et al.*, 1999; Sag & Kutsal, 2000; Tsui, 2000). This was due to the sufficient availability of binding sites of biosorbents for limited sorbate at low sorbate concentration. Whereas it leveled off eventually at high sorbate concentration as the binding sites of biosorbents had been saturated (Sag & Kutsal, 2000; Tsui, 2000). In addition, when considering the adsorption isotherms with different biosorbent concentrations of chitin B and chitosan, it was observed that PCP RC for different sorbate concentrations was independent on the biosorbent concentration. But the phenomenon from adsorption

by chitin A was quite different. Firstly, no pronounced plateau could be observed on the adsorption isotherm. That was, in the PCP concentration ranged examined, no obvious saturation had been reached. Secondly, the isotherm of lower biosorbent concentration (say 0.2 g) gave higher RC. The similar results were demonstrated by Brandt *et al.* (1997) which *Mycobacterium chlorophenolicum* PCP-1 was employed as PCP biosorbent in a low PCP concentration range (less than 50 mg/L). From these results, it indicates that chitin A possesses more binding sites than that of chitin B and chitosan. And it has potential to adsorb higher concentration of PCP (> 300 mg/L).

5.1.2.1 Modeling of biosorption

The data from adsorption isotherm was transformed into two adsorption models in order to characterize the biosorption pattern. Based on the Langmuir adsorption isotherm, two constants, affinity constant (b) and theoretical maximum adsorption capacity (q_{max}) were obtained. The affinity constant (b) reflected the relative binding tendency of sorbate. Larger value indicated a stronger bonding of sorbates towards sorbents. By comparing the results of the present study, the binding affinity of chitin A was the highest. It implied that the tendency of PCP adsorbed by chitin A was the highest, followed by chitosan and chitin B (Table 4.2). In addition, it reflected chitin A with a greatest fractional surface coverage compared with chitin B and chitosan (Sag & Kutsal, 2000). Meanwhile, the maximum adsorption capacity (q_{max}) indicated the total amount of sorbates that can be adsorbed by the biosorbent in monolayer (Lau, 2000). The higher value denoted more sorbates could be adsorbed by the biosorbent. Thus, chitin A could retain higher amount of PCP on the surface (Table 4.2).

By considering the Freundlich adsorption isotherm, two constants, adsorption capacity (k) and adsorption intensity (n), were obtained to reveal the biosorption characteristic. The adsorption capacity indicated the relative amount of sorbates that can be adsorbed by the biosorbent (Echeverria *et al.*, 1998; Lau, 2000). From the result (Table 4.2), it was obvious that chitin A could adsorb the highest amount of PCP, followed by chitosan and chitin B. Whereas the magnitude of adsorption intensity reflected the system suitability (Echeverria *et al.*, 1998; Tsui, 2000). It was suggested that when the value of n is greater than one, the biosorption was under the favorable conditions (Scott & Karanjkar, 1995; Tsui, 2000). And the

current study showed that all three biosorbents were suitable for biosorption.

However, it is requisite to count on the fitness of the models before making any conclusion. This purpose can be satisfied by considering the correlation coefficient (r^2). It could be observed that r^2 for all three biosorbents in Langmuir isotherm were high (with average 0.895, 0.989 and 0.951 for chitin A, chitin B and chitosan respectively), and prominently higher than that of Freundlich isotherm (with average of 0.558, 0.983 and 0.889 for chitin A, chitin B and chitosan respectively), especially chitin A. Therefore, the PCP adsorption on chitinous materials was better described as Langmuir monolayer model rather than Freundlich model. And based on the assumption (as described in Section 1.2.3.1), the goodness of Langmuir fit suggests that the biosorption should be a monolayer homogeneous adsorption system and there is a fixed number of sites accessible on the surface of the biosorbent (Nelson & Yang, 1995; Scott & Karanjkar, 1995; Lau, 2000; Tsui, 2000).

On the other hand, when considering the relationship between the biosorbent concentration and the constants, no strong correlation could be seen from either Langmuir or Freundlich isotherms. It revealed that the biosorbent concentration was independent on the biosorption mechanism in the experimental PCP concentration range. However, a previous study substantiated that the adsorption capacity (k) was a function of biomass concentration under the very low sorbate concentration (Brandt *et al.*, 1997). It declined significantly with the increase of PCP concentration in a biosorption system with *Mycobacterium chlorophenolicum* PCP-1 as biosorbent. Unfortunately, the reason for this different biosorption behavior is still not clear (Brandt *et al.*, 1997). But, it can show that different sorbate-sorbent system involving different adsorption mechanisms gives different interpretation from the isotherm models (Brandt *et al.*, 1997; Ning *et al.*, 1999).

5.2 Photocatalytic oxidation

It has been postulated that PCP is susceptible to mineralization by PCO (Mills & Hoffmann, 1993; Jardim *et al.*, 1997; Fong, 2001, Pecchi *et al.*, 2001). It was proved that higher than 95% of PCP RE could be reached in the range of 50 to 300 mg/L of PCP in the reaction mixture (PCP solution with TiO_2 and H_2O_2) (Fong, 2001). The higher PCP initial concentration required longer irradiation time. For example, 95% of 300 mg/L of PCP required double irradiation time (180 min) as that

of 100 mg/L of PCP (90 min) (Fong, 2001). However, few studies investigated the effect of compounds which original adsorbed on the sorbent for PCO. Thus in the present study, the semi-liquid phase of PCP was designed to investigate the influence of sorbent towards the PCO efficiency. The term "semi-liquid phase of PCP" means the soluble PCP adsorbed on the insoluble immobilizer such as biosorbent. If this semi-liquid phase PCO is proved to be feasible, it gives economic benefit, as the adsorbed PCP is no need to elute with extra solvent with additional treatment step.

5.2.1 Selection of extraction solvent

As mentioned in Section 1.1.1, PCP is water insoluble, but which is readily dissolved in organic solvents such as alcohol and ether or in alkaline solution. Therefore, the organic solvent and base solution can facilitate PCP desorption or extraction. With the reference previous studies (Matthews, 1987; Mollah *et al.*, 1996a; Gremaud & Turesky, 1997; Christov *et al.*, 1999), MeOH and NaOH were chosen as extraction solvents. Based on the results (Figure 4.16), 75% of MeOH gave the highest extraction efficiency (94%) and thus it was used as the extraction solvent in the following experiments. It was seen that the extraction efficiency increased with the higher proportion of MeOH, except 100% MeOH. This revealed that the hydrophobic MeOH was a superior medium to extract PCP. And the water (25%) was required to give protonated PCP (Equation 1.1). This neutral PCP species was much readily soluble in hydrophobic solvent.

5.2.2 Determination of hydrogen peroxide concentration

Hydrogen peroxide (H_2O_2) is one of the components for PCO reaction, as it can indirectly influence the PCO reaction rate. It can be done because it can act as an electron scavenger by reacting with electron (e^-) to produce •OH, a strong oxidizing agent (Equation 1.15). The presence of H_2O_2 can minimize the chance for recombination of the hole (h^+) and e^-; and thus allows h^+ reacted to produce more •OH (Figure 1.6). In addition, H_2O_2 can directly form •OH under irradiation by UV (Equation 1.17) (Fong, 2001; Yamazaki *et al.*, 2001). However, the principle for higher concentration of H_2O_2 giving higher performance is not always true. This is because too much H_2O_2 would react with •OH and form •OOH (Equation 5.1), and this hydroperoxide radical (•OOH) can react with •OH and further deplete the

amount of •OH and the reaction rate as well (Equation 5.2) (Akgerman & Alnaizy 2000; Fong 2001).

$$H_2O_2 + \bullet OH \rightarrow H_2O + \bullet OOH \quad (5.1)$$

$$\bullet OOH + \bullet OH \rightarrow H_2O + O_2 \quad (5.2)$$

Therefore, the concentration of H_2O_2 can influence the PCO reaction rate positively or negatively (Skurlatov *et al.*, 1997; Yamazaki *et al.*, 2001). And the concentration of H_2O_2 added should be carefully designed. From the result obtained by Fong (2001), 6.7 mM of H_2O_2 was the optimal concentration which gave the highest PCP RE. Whereas the reaction was inhibited when 33.5 mM of H_2O_2 was added (Fong, 2001). Thus, 6.7 mM of H_2O_2 was added into the reaction mixture solution initially in my current study. Then its amount was monitored and replenished if necessary. Based on the result showing the H_2O_2 consumption (Figure 4.17), it was nearly used up at about 240 min. And thus another 6.7 mM of H_2O_2 was supplied if the PCO reaction was extended beyond 4 h.

5.2.3 Effect of biosorbent concentration in PCO

Different biosorbent concentration was added for PCO in order to investigate the effect of biosorbents as well as the PCP amount on PCO reaction. It was realized that the biosorbent itself contributed the shielding effect upon UV irradiation and thus reduced the PCP degraded efficiency by PCO. In addition, the concentration of PCP could affect the PCO reaction rate and thus determined the irradiation time. In the current study, different amount of chitin A (0.4, 0.8 and 1.6 g) with PCP concentration of 0.85 mg of PCP/g of biosorbent was suspended into the reaction mixture solution for UV irradiation or suspended into ultra-pure water as control experiment (as described in Section 3.4.3).

For the control experiment, apart from evaluating the PCP loss during transfer or retained on the container, it in fact could give an insight for the movement of PCP for PCO reaction. From the result showing the distribution of PCP in control (Figure 4.18b), it could be observed that the amount of PCP on biosorbent (A_b) decreased in the first 20 min; whereas the amount of PCP in solution (A_s) increased and reached plateau after 20 min. It indicated that PCP rapidly diffused from

biosorbent into water media. Thereafter, the equilibrium was made. However, as there was no sufficient evidence to conclude there was no further movement after the experimental time. This 20 min could be only called "apparent-equilibrium" (see Section 5.1.1.3). And this implied that PCP on biosorbent released into the aqueous media rapidly once it made contact with the reaction mixture solution. And the PCO reaction was probably taken place in the solution mixture solution, but not on the biosorbent directly. Based on the recent studies (Vidal, 1997; Pecchi *et al.,* 2001; Yang *et al.*, 2001), PCO reaction was initiated on the surface of TiO_2. That meant the organic compound should adsorb on TiO_2 and allowed •OH to attack (see Section 1.3.2). If this principle is absolute, PCP should firstly desorb from biosorbent into the reaction mixture solution, and then adsorbed on TiO_2 surface in the reaction mixture and initiated the PCO reaction. And this speculation can interpret the result showing the distribution of PCP in the PCO reaction (Figures 4.18a, 4.19a and 4.20a). The relative high concentration of PCP appeared in the reaction mixture (A_s) at initial stage implied that PCP on biosorbent had released into aquatic media rapidly. Then PCP in reaction mixture was adsorbed by TiO_2 and degraded. This interrupted the PCP equilibrium between the biosorbent and surrounding solution. Thus the biosorbents acted as PCP reservoir to recruit that in the mixture solution. Therefore, it led to the sharp decline of A_b but relatively constant for A_s. If this proposed mechanism is valid, the rate determining factor should be the PCP diffusion rate from biosorbent into aquatic media, and the PCP equilibrium concentration.

The degradation efficiency (Figure 4.21) and degradation capacity (Figure 4.22) were calculated from $A_b + A_s$, the results were similar with the previous study of liquid phase PCP for PCO (Fong, 2001). The PCO rate increased with decreasing amount of PCP (1.36, 0.68 and 0.34 mg of PCP on 1.6, 0.8 and 0.4 g of biosorbent). And all three had reached the highest DE (around 100%) at 4 h. It indicated that PCO of PCP still occurred in spite of the presence of biosorbent in the PCO system. The biosorbent only decreased the RE rate of PCP, as time was required for PCP to diffuse from the biosorbent into the reaction mixture for PCO. But the shielding effect of biosorbent did not significantly mask the PCO reaction.

5.2.4 Effect of PCP amount on biosorbent in PCO

The previous section mainly investigated the effect of biosorbent in PCO reaction. All biosorbent consisted of the same amount of PCP (0.85 mg of PCP/g of biosorbent). In this section, the effect of PCP amount on biosorbent was evaluated. This time, the biosorbents containing two concentrations (0.85 and 6.50 mg of PCP/g of biosorbent) were utilized in PCO for 60 min. By considering PCP DE (Figure 4.23a), it could be observed that both concentrations showed similar results without statistical difference. This revealed that the amount of PCP on biosorbent did not influence the performance of PCO. And combining the results from these two sections, it could be concluded that both the amount of biosorbents as well as PCP seemed to have no obvious inhibition on PCO.

5.2.5 Determination of chloride ion concentration and total organic carbon during PCO

In order to prove the complete mineralization of PCP, analyzing chloride ions concentration and total organic carbon (TOC) were usually employed (Mills & Hoffmann, 1993; Jardim *et al.*, 1997). The chloride ions concentration can reflect the degree of dechlorination of PCP. The amount of chloride ion increased continuously (Figure 4.24a) in the PCO reaction within the experimental time interval, even higher than the theoretical amount (Table 4.3). Whereas for Cl^- concentration in control experiment (Figure 4.24b), some Cl^- was detected in the solution. Based on the result of DE on control experiment (Figure 4.21), no PCP should be degraded in control. Therefore, it showed that the additional Cl^- was originally presented in the system, which was not contributed from the dechlorination of PCP. This might come from the crustacean shell or contamination during the production process. Also, some Cl^- was added and adsorbed on the biosorbent during the biosorption procedure as HCl was required for pH adjustment. Therefore, the measurement of Cl^- could not reflect the dechlorination of PCP.

In the PCO reaction, PCP, the organic compound, should be cleaved. This can be monitored by the measurement of total organic carbon (TOC). The removal of TOC indicated the mineralization of PCP. However, in the present study, TOC in the PCO reaction had not been removed, but increased continuously (Figure 4.25a). This unexpected result might be due to the degradation of protein on biosorbent by PCO. From Table 4.4, it showed that there was 21% of protein presented in chitin A;

whereas, 16% of it was degraded after PCO. However, the protein were not easily released out if no UV irradiation, as TOC in control remained constant. Therefore, during PCO, not only PCP but also the biosorbent underwent the mineralization. Therefore, the result of TOC could not reflect the mineralization of PCP. And thus, it showed that both TOC and chloride ions concentration measurement could not give clear picture on the fate of PCP during PCO.

5.2.6 Identification the intermediates of PCP degradation by PCO

To realize the fate of PCP for PCO reaction, another direct method can be applied. It is to identify the transient species of PCP in the solution mixture, which can be achieved by the analysis of GC/MS. In the present study, the possible intermediates of PCP at 60 min were 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP) and tetrachlorohydroquinone (TeHQ) (Figure 4.26). This result was agreed with some previous studies (Mills & Hoffmann, 1993; Jardim *et al.*, 1996; Fong, 2001) (Table 1.12). As the *para*-position had less steric hindrance effect, •OH tended to attack the chlorine first by either dechlorination or oxidation (Mills & Hoffmann, 1993). Therefore, 2,3,5,6-TeCP and TeHQ are the major transient species of PCP for PCO. Meanwhile, other than these two, tetrachlorobenzoquinone (TeBQ) is another major intermediate usually identified in many studies (Mills & Hoffmann, 1993; Jardim *et al.*, 1996; Fong, 2001). However, it could not be found in the present study. This might be due to the low concentration presented in the reaction mixture. It was reported that TeBQ could be reduced rapidly that its concentration was too low to be detected (see Figure 1.7), as the detection limit of this experiment was only 1 μg/L.

5.2.7 Evaluation of the change of PCO treated biosorbents

To select a suitable method to remediate PCP, economics is one of the important factors to be considered. Though the cost of chitin is quite low (see Section 1.2.1.3), it increases the operation cost if the biosorbent cannot be reused and required to exchange frequently. Therefore, it is necessary to investigate whether the biosorbent is suitable for reuse. To achieve this purpose, the chitin assay, DRFT-IR and protein assay were employed to evaluate any structural change of biosorbents. Whereas the comparison of biosorption efficiency of PCO treated and untreated biosorbents was done to judge the efficiency change of biosorbents.

5.2.7.1 Chitin assay

It is reminded that chitin B is the demineralizating and deproteinating product of chitin A (Figure 1.5). The chitin content in chitin B should be much higher than that of chitin A, and which is termed as "pure chitin". From the result (Table 4.4), the chitin content of chitin B was 91%. Some impurities still remained attach in chitin B. Whereas for chitin A, other than chitin, some protein, carbonate salt and lipids might be presented. Therefore, the chitin content was lower (72%). By considering the untreated and PCO treated biosorbents, it could be observed that the chitin contents were not significantly different. It indicated that the chitin was resistant to degradation of PCO.

5.2.7.2 Diffuse reflectance Fourier transform infra-red spectroscopy

To examine the structure of chitinous materials, DRFT-IR spectroscopy can be employed. From Figure 4.27, it was clear that each biosorbent showed similar spectra of untreated and PCO treated (Figures 4.27a, 4.27b and 4.27c). No prominent peaks were developed or disappeared after PCO treated. Thus, it could be concluded that the PCO treatment did not have prominent effect on the structure of biosorbents, even for 8 h irradiation time (Figure 4.28).

In addition, the spectra could tell the functional groups of the biosorbents (Tsui, 2000; Pecchi *et al.*, 2001; Yang *et al.*, 2001). Table 5.1 summarized the infrared characteristic group frequencies of functional groups presented in chitinous materials. The low transmittance at some wavenumbers of the spectra indicated that the functional groups with the correspondent vibration frequencies were usually presented, though the vibration frequency was largely affected by the surrounding functional groups. Both amide I and II bands represent the residual —CONH— groups. Amide I band (1670 to 1650 cm^{-1}) is responsible for the C=O stretching vibration, and amide II band (1570 to 1515 cm^{-1}) is due to a motion combining both the N—H bending and the C—N stretching vibrations (Socrates, 1994). These two bands are known to become progressively weaker with the increased deacetylation of chitin. That is, the infra-red spectrum of chitosan gives weaker absorption bands at those wavenumbers (Pangburn *et al.*, 1984; Tsui, 2000). In addition, at 1600 cm^{-1}, a band assigned to water also appeared (Pecchi *et al.*, 2001).

Table 5.1 The infra-red characteristic group frequencies for the stretching vibrations of functional groups presented in chitinous materials (Socrates, 1994).

Functional groups		**Regions (cm^{-1})**
Methyl ketones	$\geq C=O$	3550 – 3220; 1360 – 1355
Saturated primary alcohol	$-CH_2-OH$	1085 – 1030
Primary aliphatic amines	$\geq CH-NH_2$	1240 – 1170; 1040-1020
Secondary aliphatic amine	$-CH_2-NH-CH_2-$	1145-1130; 1190-1170
Amine	$-NH_3^+$	3350-3100; 2500
Secondary amides	$\begin{matrix} O \\ \| \\ -C-NH- \end{matrix}$	
: Amide I		1670 - 1650
: Amide II		1570 - 1515

5.2.7.3 Protein assay

As mentioned in Section 1.2.1.3, protein is one of the components in shrimp shell, it is worthy quantifying the protein presented in the biosorbents in current study in order to further understand the characteristics of the biosorbents. In addition, the protein assay could provide evidence whether PCO can degrade it.

From the result (Table 4.5), it was clear that chitin A contained the most protein (21.09%). It was reasonable as chitin A was the crude grounded product of shrimp shell. After deproteination by dilute NaOH, a large amount of protein residues from chitin A was removed and this made chitin B consist of very little amount of protein (1.12%) (Figure 1.5). Whereas in the process of deacetylation, concentrated NaOH was applied to the biosorbent, which further diminished the protein content of biosorbent, chitosan (0.73%). Meanwhile, it could be observed that the protein content of biosorbents was greatly decreased after PCO treated. In other words, the proteins present in biosorbents were susceptible to the degradation of PCO.

5.2.7.4 Biosorption efficiency

After discussing the structural change of biosorbents after PCO treatment, it is time to investigate the practical change. The untreated and PCO treated biosorbents were utilized for PCP biosorption under the same conditions in order to see any efficiency change. It is important not only it can tell the difference from the practical point of view, but also it can reflect the component responsible for biosorption indirectly. From the aforementioned three analysis, it was shown that both chitin contents and major functional groups of biosorbents had no significant change; whereas the protein contents was greatly reduced after PCO. Thus we could induce whether protein is important for biosorption merely from the biosorption performance of PCO treated biosorbents. From the result (Figure 4.29), It could be realized that the biosorption efficiency of each biosorbent had no significant difference, though a slightly higher efficiency of untreated chitin A was observed. Therefore, it revealed that protein might not be the important component for biosorption of PCP; even it was effective for metal ion binding (Sag & Kutsal, 1995; Tsui, 2000). Meanwhile, this speculation just rejects the inference that the impurities in chitin A might contribute to the PCP biosorption mentioned in Section 5.1.1.1.

Therefore, the superiority of chitin A for PCP biosorption might be merely due to the larger surface area and less degree of depolymerization.

5.2.8 Multiple biosorption and PCO cycles of PCP

After proving the biosorbents without change in efficiency after PCO, it preliminarily induces that they can be reused after biosorption and PCO. And it is necessary to investigate the repeated reusability. Two concentrations of chitin A (0.8 and 1.6 g) were utilized, because it could give a more supportive result rather than only one set of experiment. To evaluate the ability for multiple PCP biosorption and PCO cycles, the PCP adsorbed biosorbent was reacted in PCO for 4 h. This irradiation time was fixed as almost all PCP was removed and no more H_2O_2 was required to recruit. It was more convenient for the practical use.

The multiple cycles for two biosorbent concentrations were similar. The RE for all four multiple cycles were all approaching especially the one for 1.6 g of biosorbent (Figure 4.31). This implied that the biosorbent was still effective even after four biosorption and PCO cycles. And therefore, it cannot be reused for at least four times without replacing the new biosorbent. This can make the system more economic and convenient for continuous used.

5.2.9 Evaluation for the toxicity change of PCP adsorbed biosorbents during PCO

To evaluate the toxicity of samples, Microtox® test is popular employed as it possesses a lot of advantages (Bolduc & Anderson, 1997; Cauntú *et al.*, 2000). Firstly, the use of significant numbers of standardized test organism, *Vibrio fisheri*, makes the test highly precise, reproducible, reliable and representative all over the world. Also, the test is fast which the result can be obtained within a short period of time, 5 to 15 min. From the previous study, the EC50-5min and EC50-15min of PCP on Microtox® test were 0.65 and 0.39 mg/L (Fong, 2001). And it showed increasing EC50 of PCP along the time of PCO. That was, PCO could contribute in detoxification (Muir & Eduljee, 1999; Fong, 2001). However, this custom toxicity test was usually based on the aqueous phase PCP. It could not reflect the toxicity of the present study, as the PCP was bounded on biosorbent, only less released into the aqueous media. Therefore, the solid phase Microtox® test was employed instead of

basic test.

For solid phase Microtox® test, the bacteria were allowed to respond in the presence of weighed biosorbent. The PCP as well as the other materials on the biosorbent might contribute for the toxicity. With the reference of EC50 of untreated and PCO treated chitin A without PCP, the toxicity of PCP adsorbed biosorbent changing along the irradiation time for PCO could be compared. It could be seen that the toxicity of PCP adsorbed biosorbent of control remained at high level (690-331 mg/Kg); whereas it decreased continuously along the PCO treatment time and even lower than that of untreated chitin (Table 4.6). It indicated that PCO could thoroughly detoxify the biosorbent, not only the PCP, but also the toxic materials presented on biosorbent. And it gave evident that even the biosorbent was not subjected to reuse, it was safe to be disposal.

6. Conclusion

In the present study, the target pollutant, pentachlorophenol (PCP), was treated by a two-step remediation method, biosorption by chitinous materials followed by photocatalytic oxidation (PCO). The results of these two parts of experiments could be summarized as following:

- **Batch biosorption experiment:**
  - The PCP removal efficiency (RE) increased under acidic condition, since the uncharged PCP, mostly appeared at low pH (<4.74), favored to adsorb on organic matter.
  - The higher biosorbent concentration, the higher PCP RE was, as more binding sites were provided for PCP. But it gave lower PCP RC since the high concentration of biosorbent brought a screening effect on the outer layer of biosorbents, and hence protected the binding sites from PCP.
  - The PCP biosorption on chitinous materials were described as the typical biphasic process, the first rapid step followed by a slow step. The first rapid step was due to the sufficient availability of binding sites, whereas the latter step might be attributed to the steric hindrance between the adsorbed and unadsorbed sorbates. The apparent-equilibrium time in the present study was found to be 60 min.
  - The PCP adsorption decreased with increasing temperature. This induced that the biosorption mechanism was due to the exothermic force, e.g. hydrogen bonding. In addition, the high temperature could favour the PCP desorption.
  - Higher agitation rate resulted in better PCP biosorption due to better contact of the sorbates and sorbents.
  - In the batch isotherm studies, the increase of the initial PCP concentration showed higher PCP RC especially at low PCP concentration, as for the higher probability of contact between sorbates and sorbents. And it seemed that the PCP biosorption on chitin B and chitosan reached saturated at high PCP concentrations owing to the limited supply of binding sites. But this phenomenon did not appear on chitin A at the experimental PCP concentration (5-300 mg/L).

- The PCP adsorption on all three biosorbents showed better fitted with Langmuir adsorption isotherms rather than Freundlich one. It implied that the biosorption should be due to the homogeneous monolayer.
- By considering the performance of three biosorbents to the effect of varying different experimental conditions and the constants obtained from two monolayer models, chitin A showed the highest efficiency in removing PCP, followed by chitosan and chitin B.

■ **Photocatalytic oxidation (PCO):**

- The biosorbent concentration (0.4, 0.8 and 1.6 g in 100 mL of reaction mixture solution), which was susceptible to PCO, did not significant affect the performance for PCO of PCP. The high biosorbent concentration only reduced the PCP degradation rate, but after 4 h of irradiation time, almost all PCP was degraded.
- There was no statistically difference on the PCP degradation efficiency for different amount of PCP on biosorbent (0.85 and 6.5 mg/g of biosorbent).
- 2,3,5,6-Tetrachlorophenol and 2,3,5,6-tetrachlorohydroquinone were the major intermediates of PCP in the present study.
- PCO could detoxify the PCP as well as the toxic materials released from the biosorbent, based on the results of Microtox® test.
- Some fraction of proteins from chitin A were degraded by PCO. But it was not attributed to the PCP adsorption.
- Chitin was resistant to PCO degradation and thus it could be used for multiple PCP biosorption and PCO cycles for at least four times without decreasing the PCP biosorption efficiency.

In accordance with the above summarized results and the background information, chitin A, the crude chitin manufactured from shrimp shell, can be used as an effective and economic biosorbent to remove PCP from water, compared with the pure chitin (chitin B) and chitosan. This might be attributed to the higher amount of binding sites available for PCP and lower degree of depolymerization in chitin A. It is noteworthy that the more steps in the production process of biosorbent, the higher the cost of the treatment and the higher degree of depolymerization. In the production of chitin A, only 10% of solid waste is produced from shrimp shell

(*Penaeus japonicus*). Whereas to produce pure chitin and chitosan, more steps including deproteination, demineralization and deacetylation are involved with the consumption of large amount of HCl and NaOH, and 70% and 80% of waste are produced respectively in results. The process does not only increase the cost of treatment, but also increases the degree of depolymerization of biosorbent. Therefore, based on the results of efficiency, economic and environmental safety, chitin A is highly recommended as a biosorbent for wastewater treatment rather than extracted pure chitin or chitosan.

On the other hand, the semi-liquid phase PCP seems feasible for PCO degradation. It is glad tidings as it can cut down the step and the cost for eluting PCP from biosorbent. In addition, it approves the feasibility to combine the two remediation process, biosorption and PCO. Biosorption is the phase-transfer of the pollutant; whereas PCO is to completely degrade the toxic compounds. This two-step process makes the treatment more complete and capable to thoroughly mineralize PCP. On the contrary, the chitin is proved to be resistant to PCO. It can be used for multiple biosorption and PCO cycle. This further lowers the cost of treatment and fulfills the criteria of wastewater treatment – economic and efficiency. Therefore, further investigation is worthwhile in order to yield the higher efficiency after optimization and show the feasibility of this two-step treatment for other pollutant and sorbent combination.

7. Recommendations

It is realized that the two-step treatment, biosorption by chitinous materials followed by PCO, is feasible for PCP remediation. In order to increase the industrial applicability, further studies are suggested to be investigated, which are listed below.

Firstly, it is shown that the RE of PCP can be enhanced under buffering system. However, it might not be economically feasible to administrate a large amount of buffer solution into the pilot-scale treatment. Therefore, another suggestion to improve the RE of chitin is the chemical modification of chitin (Thome & Jeuniaux 1997; No & Meyers, 2000). The chitinous materials can be modified by cross-linked with glutaraldehyde or synthesis of highly porous beads of chemically cross-linked chitin. This can increase the resistance of chitinous materials to solubilization in acidic pH effluents. In addition, it can advance the functional groups available for adsorption. Thus, the PCP RE can be improved.

In addition, the treatment is more applicable in the dynamic condition as it can handle a huge amount of wastewater in a smaller area. This can be achieved by the column operation fixing with the chitinous material. Further study of the efficiency preformed by column-immobilized reactors for continuous removal of toxicants should be directed (Guibal *et al.*, 1999; Ning *et al.*, 1999; No & Meyers, 2000; Tsui, 2000).

Furthermore, in the present study, the PCO condition was selected with the reference of previous study, which optimized the RE of liquid phase PCP by PCO (Fong, 2001). Meanwhile, the condition of liquid and semi-liquid phase PCP is different. Research should be directed toward further studies of optimalization of PCO for semi-liquid phase PCP, including pH, H_2O_2 and TiO_2 concentrations, in order to improve the PCP DE from PCO.

The pilot-scale studies are recommended to apply the two-step treatment to actual waste streams, since levels of organic or inorganic compounds present in discharge stream might vary the performance of biosorption as well as PCO (No & Meyers, 2000).

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